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ACTA PÆDIATRICA SCANDINAVICA

VOL 66/NOV 1977/NO 6

ISSN 0001-656X

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◄ *Subscription*

ACTA PÆDIATRICA SCANDINAVICA

Subscription and Distribution
The Almqvist & Wiksell Periodical Company
26 Gamla Brogatan
S 101 20 Stockholm 1, Sweden

Acta Pædiatrica Scandinavica publishes papers in English on scientific investigations in the field of paediatrics Each *issue consists of about 100* pages six numbers forming one volume The numbers will be issued every second month *Supplements* will be delivered to subscribers free of charge

Acta Pædiatrica Scandinavica will consider papers from authors in all countries Articles are accepted *for publication on condition that they* are contributed solely to this journal and have not been published elsewhere previously Original articles should report scientific findings within the paediatric field of interest Review articles should contain an objective account *of different points of view and an internationally* representative bibliography Case reports should contain accounts of rare syndromes new diagnostic tools and methods new kinds of treatment and laboratory research with foreseeable practical application Letters to the Editor is intended to be a forum for comments on current literature short discussions and short preliminary communications which are thought to be important and urgent enough to warrant rapid publication

All papers are subject to editorial revision to make them conform to the style adopted by Acta Pædiatrica Scandinavica A paper should not exceed 6 printed pages, *including space for figures* and tables Exceptions are occasionally allowed but the author will be charged for the exceeding pages Tables and illustrations covering more than three pages in original articles and two pages in Case reports are also printed at the author s expense Monographs and more extensive articles may be published separately as Supplement at the author s own expense

Acta Pædiatr Scand 66 665–672 1977

USE OF WEANING FOODS (BEIKOST) IN AN INDUSTRIALIZED SOCIETY

Socio economic and Psychological Aspects

EVA MARI KÖHLER LENNART KÖHLER and BERTIL LINDQUIST

From the Department of Paediatrics University Hospital Lund Sweden

ABSTRACT Kohler E M Kohler L and Lindquist B (Department of Paediatrics University Hospital Lund Sweden) Use of Weaning Foods (Beikost) in an industrialized society Socio-economic and psychological aspects Acta Paediatr Scand 66 665 1977.— An interview was performed among mothers of 466 children aged 6–18 months in order to elucidate which factors influence the parents choice of industrially produced or home made foods when feeding their infants with Beikost The use of canned foods was more popular among mothers of very young children the families own table food being considered as nutritionally not adequate and also less convenient The home-cookers on the other hand were convinced that industrially prepared Beikost was not necessary from nutritional taste or economical aspects Feeding problems occurred in about 15% of all families irrespective of feeding practices Social and economic factors like education working and money spent on food seemed to influence the families choice of food for the children to a very small extent implying that the way of feeding children had no deeper impact on their standard of living Instead the mother s own activities interest knowledge and ideas of how to feed children were decisive for her choice the home cookers were more interested in household work like shopping baking and cooking and these activities did not exhaust them as much as they did the jar buyers Information from the food industries was the most important source for the parents knowledge about nutrition and feeding of babies irrespective of their feeding practices After knowledge due to experience from older children the Child Health Centres were the third most appreciated source of information It is therefore most important that the baby food industries give information of high quality and that they follow certain ethical principles preferably formulated by paediatricians acting as medical consultants to the industry However the primary responsibility for information to parents about baby food falls on the country s health authorities Considering the apprehension of feeding management often revealed by the mothers and also the relatively high frequency of feeding problems reported it seems wise to educate and support the Child Health Centres towards a more active participation on nutritional matters

KEY WORDS Weaning foods feeding practices Beikost baby food industry

The consumption of industrially produced baby food in Sweden has increased dramatically during the last decades In 1975 the average Swedish infant consumed about 70 litres of starting infant formulas (used from birth up to 4–5 months of age) and about 600 jars of solid and semisolid baby food (so called Beikost)[1] (12) (Fig 1)

From every point of view it is obvious that breastfeeding is the best feeding system for babies and this fact is no doubt accepted also by the baby food industries today It is also generally and officially accepted that home made infant formulas cannot assure the infant the same adequate nutrition as can the industrially produced formulas (3)

[1] In this calculation it is assumed that Beikost is entirely consumed by infants This is however not true to some extent it is also consumed by toddlers and by older persons

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The consumption of industrially produced baby food in Sweden has increased dramatically during the last decades In 1975 the average Swedish infant consumed about 70 litres of starting infant formulas (used from birth up to 4–5 months of age) and about 600 jars of solid and semisolid baby food (so called Beikost)[1] (12) (Fig 1)

From every point of view it is obvious that breastfeeding is the best feeding system for babies and this fact is no doubt accepted also by the baby food industries today It is also generally and officially accepted that home made infant formulas cannot assure the infant the same adequate nutrition as can the industrially produced formulas (3)

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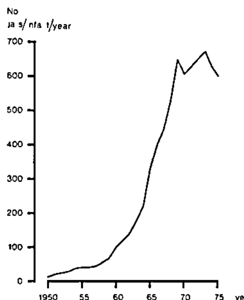


Fig 1 Consumption of baby food in jars in Sweden expressed as number of jars per infant and year (It is assumed that all consumption is made by infants the consumption by others e g older people is disregarded)

However the wisdom of giving industrially produced Beikost has been questioned It has been argued that these products contain poisons (e g nitrates biocides) that they contain unnecessary additives (colours etc), that vital ingredients e g vitamins are destroyed during the processing operations and furthermore that the taste and consistency of the food are too weak and soft to teach the children the sensation of real food in the mouth

The industry scientists and the nutritional physiologists have refuted these arguments by stating that the industries careful selection of the best raw products as well as the rigorous control and surveillance of the production lines are in fact the best guarantee for high quality and poisonfree baby food They also mean that the selection possibilities between various foods and the convenience of having a product in a jar ready when the child is hungry makes life much easier for the parents and for the child

Exactly which motives lie behind the parents selection of way of feeding are not known which people prefer to use canned baby food—and ctors influence their choice—in comparison to those preferring home made food? How important is the professional advice from the Child Health Centres in establishing the children s eating habits?

In order to elucidate these and other factors of socio economic and psychological nature which might influence the parents way of feeding their infants and toodlers with Beikost a study was performed interviewing mothers in a health district in Southern Sweden

MATERIAL

In the district consisting of one industrial town surrounded by small villages and countryside all 624 children aged 6–18 months were selected from the county population register Children with parents who did not speak or understand Swedish were excluded from the study (36 families) Another 39 families had moved from the area Of the remaining 549 families 83 (15 1%) had no time or refused to cooperate Thus 466 children 256 in the town and 210 in the villages and in the countryside participated in the study The age of the children were as follows 74 6–10 months 65 10–12 months and 327 12–18 months of age

METHODS

After arranging an appointment a trained interviewer visited the families homes and performed the interview which consisted of about 75 questions and took about 1 hour The interviewer was very well received and most of the mothers seized the opportunity to talk lengthy and *freely to someone who was genuinely interested in their* babies and their everyday small problems

RESULTS

Feeding practices

The frequency of breastfeeding was somewhat higher than in Sweden as a whole (Fig 2) When breastfeeding ceased all children were given industrially produced infant formulas from a bottle

The introduction of Beikost in Sweden is recommended at 3–4 months of age (3) In the present material it occurred in 73% at this age and at the age of about 6 months one meal of breastmilk/formula was replaced by Beikost in about two thirds of the children according to general recommendations (3) By then the

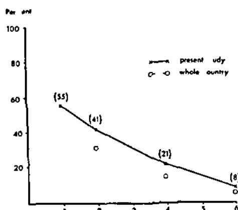


Fig 2 Totally breastfed children Figures for whole country represent official statistics in Sweden for 1970

majority preferred to give industrially produced Beikost Only 11% trusted their own cooking ability enough to start with home made Beikost as the only solid food

Among the families studied three groups were found with different feeding practices One group—the largest one of the whole material—preferred home made food only the home cookers (231 children) another group gave only industrially produced foods the jar buyers (65 children) and a third group gave both kinds of food either at the same meal or on different days group mixed (170 children) (Table 1) There was however a marked age dependency the use of jars being more popular the younger the child was

Table 1 *Feeding practices with respect to beikost in 466 children of different age groups*

Age in months	Per cent Home cookers (n=231)	Jar buyers (n=65)	Mixed (n=170)	Sum (n=466)
6–10	16 2	55 4	28 4	15 9
10–12	29 2	21 5	49 3	14 0
12–18	61 2	3 1	35 7	70 1
Sum	49 6	13 9	36 5	100 0

Home made food

The reasons for giving home made food tabulated in Table 2 were dominated by the conviction that specially prepared solid or semisolid foods for children were not necessary The family s own food was cheaper (39 2%) better (31 9%) and more tasteful (26 9%) than canned food The children s dislike of canned foods was also a factor of some importance more in older children than in younger ($p<0.01$)

Canned food

Parents giving canned food did it because they felt that their own food was not adequate for their children or that the family s eating time was not suitable (46 0% and 30 2% respectively) (Table 3) The matter of convenience was also important for families using

Table 2 *Reasons for giving home made food in per cent (Home cookers and Mixed)*

	Age of children 6–10 months (n=33)	10–12 months (n=51)	12–18 months (n=317)	Sum (n=401)
No need for special food for the child	63 6	56 9	57 4	57 9
Cheaper	33 3	37 3	40 1	39 2
Better nutrition	33 3	7 5	32 5	31 9
Canned food too dull and tasteless	4 2	21 6	28 1	26 9
The child does not like canned food	3 0	0	13 9	11 2
Canned foods contain additives and preservatives	1 1	9 8	9 1	9 5
I like cooking"	3 0	3 9	3 8	3 7

Table 3. *Reasons for giving canned foods in per cent ("Jar buyers" and "Mixed")*

	Age of children			
	6–10 months (n=62)	10–12 months (n=46)	12–18 months (n=127)	Sum (n=235)
Family's food not adequate	30.6	37.0	56.7	46.0
More convenient	50.0	52.2	37.0	43.4
Family's eating time not suitable	25.8	30.4	32.3	30.2
Better nutrition	40.3	37.0	22.0	29.8
Faster	16.1	15.2	18.1	17.0
Other person can give the foods	8.1	10.9	12.6	11.1
Uncertain how to cook	12.9	6.5	4.7	7.2
Cheaper	0	0	0	0

canned foods: for 43.4% it was more convenient in a general sense, 17% said it was faster and 11.1% % considered it an easy way to let other persons than the mother feed the child.

Better nutrition through feeding the children with industrially produced foods was an argument used by 29.8%, more frequently for the young children than for the older ones ($p<0.01$). No one regarded it cheaper to use canned foods.

Feeding problems

All three groups were equally satisfied with their way of feeding their children, although about 15% in each group were worried that the children did not get the proper nutrition (Table 4). The frequency of feeding problems

Table 4. *The mothers' knowledge of nutrition and their satisfaction with the children's appetite and weight gain*

	Per cent				Statistical difference between the groups
	Home cookers (n=231)	Jar buyers (n=65)	Mixed (n=170)	Sum (n=466)	
Knowledge					
Very uncertain in nutritional matters	0.4	1.5	1.2	0.9	NS
Rather uncertain in nutritional matters	11.7	16.9	17.1	14.4	
Not at all uncertain in nutritional matters	87.9	81.6	81.7	84.7	
Appetite					
No feeding problems	82.3	83.1	81.8	82.2	NS
Problems now and then	14.3	9.2	11.8	12.7	
Always problems	3.5	7.7	6.5	5.2	
Eat too little	12.1	6.2	9.4	10.3	
Eat only certain dishes	1.3	1.5	4.1	2.4	
Eat too much	2.6	4.6	4.7	3.6	
Weight gain					
Satisfying weight gain	84.0	89.2	71.8	80.3	$p<0.001$
Too slow weight gain	7.8	4.6	15.9	10.3	
Too rapid weight gain	8.2	6.2	12.4	9.4	

Table 5 *Feeding practices and social factors*

	Per cent				Statistical difference between the groups
	Home cookers (n=231)	Jar buyers (n=65)	Mixed (n=170)	Sum (n=466)	
Mother's education					
Basic	33 3	29 2	32 9	32 6	NS
Secondary	54 1	55 4	52 4	53 7	NS
High school	10 8	9 2	11 2	10 7	NS
Academic	1 7	6 2	3 5	3 0	NS
Father's education					
Basic	4 7	21 5	24 1	24 0	NS
Secondary	55 8	47 7	56 5	54 9	NS
High School	9 1	16 9	5 3	8 8	NS
Academic	5 2	9 2	9 4	7 3	NS
Mother's working					
At home only	42 0	53 8	41 2	43 4	NS
Full time outside home	5 5	21 5	21 2	23 4	NS
Part time outside home	32 5	24 6	37 6	33 3	NS
Single mother	4 3	4 6	4 7	4 5	NS
Only child	61 5	53 8	48 2	55 6	p<0 05
Care of child during the day					
At home	61 0	75 3	55 3	60 9	p<0 001
Child eats only at home	62 3	73 8	56 4		p<0 05
Supply at home					
Washing machine	74 0	83 1	73 5	75 1	NS
Drying machine	25 1	47 7	31 8	30 7	p<0 01
Irone machine	29 9	46 2	27 6	31 3	p<0 05
Refrigerator	99 6	93 8	99 4	98 7	NS
Vacuum cleaner	98 7	100 0	99 4	99 1	NS
Freezer	93 1	92 3	86 5	90 6	NS
Colour TV	5[illegible] 4	50 8	55 9	53 4	NS
Money spent on food (Sw Kr / week)					
[illegible]00	15 6	20 0	18 2	17 2	
[illegible]00–300	51 9	40 0	44 1	45 3	
[illegible]300	16 0	15 4	12 4	14 6	
do not know	16 5	4 6	25 3	20 8	NS
Social contacts					
Regular contact with friend	63 6	70 8	74 7	68 7	
Sometimes	15 6	10 8	9 4	12 7	p<0 05
Seldom	0 8	18 5	15 9	18 6	
Mothers very tired from daily work	9 5	12 3	6 5	8 8	p<0 001
Activities inside and outside home					
Mother active (clubs etc)	40 7	9 2	34 1	36 7	NS
Father active (clubs etc)	39 0	50 8	42 4	41 8	NS
Always carefully planning the shopping	54 1	30 8	45 3	47 6	p<0 01
Baking at home	49 8	6	9 4	39 1	p<0 001

Table 3 *Reasons for giving canned foods in per cent (Jar buyers and Mixed)*

	Age of children			
	6–10 months (n=62)	10–12 months (n=46)	12–18 months (n=127)	Sum (n=235)
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More convenient	50 0	52 2	37 0	43 4
Family s eating time not suitable	25 8	30 4	32 3	30 2
Better nutrition	40 3	37 0	22 0	29 8
Faster	16 1	15 2	18 1	17 0
Other person can give the foods	8 1	10 9	12 6	11 1
Uncertain how to cook	12 9	6 5	4 7	7 2
Cheaper	0	0	0	0

canned foods for 43 4% it was more convenient in a general sense 17% said it was faster and 11 1% % considered it an easy way to let other persons than the mother feed the child

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	Home cookers (n=231)	Jar buyers (n=65)	Mixed (n=170)	Sum (n=466)	
Knowledge					
Very uncertain in nutritional matters	0 4	1 5	1 2	0 9	NS
Rather uncertain in nutritional matters	11 7	16 9	17 1	14 4	
Not at all uncertain in nutritional matters	87 9	81 6	81 7	84 7	
Appetite					
No feeding problems	82 3	83 1	81 8	82 2	NS
Problems now and then	14 3	9 2	11 8	12 7	
Always problems	3 5	7 7	6 5	5 2	
Eat too little	12 1	6 2	9 4	10 3	
Eat only certain dishes	1 3	1 5	4 1	2 4	
Eat too much	2 6	4 6	4 7	3 6	
Weight gain					
Satisfying weight gain	84 0	89 2	71 8	80 3	p<0 001
Too slow weight gain	7 8	4 6	15 9	10 3	
Too rapid weight gain	8 2	6 2	12 4	9 4	

age (2) However in toddlers and older infants home made foods accounted for a larger part of the Beikost as compared to younger infants thus in age group 12–18 months 61 2% got exclusively home made foods (Table 1) This is in agreement with the feeding practices in the United States (2)

Parents who preferred industrially produced Beikost were convinced that this was generally most adequate for the child although aspects of convenience also played an important role On the other hand the home cookers thought that their own table food was as adequate or nutritionally even better than that bought in jars It is interesting to note that 9 5% still believe that the industrially produced baby food contains harmful additives and preservatives in spite of heavy information campaigns by the industry on this matter

Estimates of intakes of calories and different nutrients were not made in this study but we have every reason to believe that no deficiency in these matters exists Studies on child health and nutrition in other areas both nearly and far away have all confirmed that the general health condition of children in Sweden is very good they are taller and heavier than children of corresponding age in other countries the haemoglobin concentration is generally satisfying only few have mild anemia and the nutritional requirements are well covered (5 6 7 8 9) Feeding problems still occur but do very seldom depend on lack of food Instead they exist among children who eat too little according to their parents opinion i e problems that are based on an emotional and behavioural level (4 10 11)

Such problems were reported in the present study too but with no consistent association with feeding practices

The fact that social and economic factors to a very small extent seem to influence the choice of food for the children may imply that the economic situation among these families was so good that they could allow themselves a freedom of choice without deeper impact on their standard of living Instead it seems that the mother s own activities interest knowledge and ideas of how to feed children are decisive for her choice

So it is obvious that although the home cookers had a less complete supply of household machines they were more interested in household work like shopping baking and cooking and this did not exhaust them as much as it did the jar buyers

Information about baby food and nutrition is offered to parents from many quarters today both private and offcial ones Best resources in these matters are owned by the food industries Their information reach their customers by advertising and publicity campaigns in magazines and shops as well as by pamphlets sent directly to parents and furthermore also through professionals As a result information given by the food industries is the parents dominating source of knowledge about their children s nutrition and about feeding practices Even parents who preferred home made food used the food industries as their main source of information Under these circumstances it is of course most important that the information and the sales promotion activities by the baby food industries are of a very high quality and that they follow certain basic ethical principles These should protect the parents not only from getting false and biased facts but also against unduly persuasive advertising In a few countries e g in Sweden paediatricians as medical consultants to the industry have already formulated such principles (Medical Standards for Marketing of Infant Foods) which have been accepted by the industry (1) In other countries the development of such principles is in progress

Nevertheless information about nutrition and feeding practices should not be left solely to the industries no matter how good it is The primary responsibility for such information lies on the country s health authorities which consequently should make non commercial information material available The Child Health Centres have a traditional and

Table 6 *Sources of information about nutrition and feeding practices of babies*

Sources of information	Per cent Home cookers ($n=231$)	Jar buyers ($n=65$)	Mixed ($n=170$)	Sum ($n=466$)	Statistical difference between the groups
Baby food industry	46.8	76.9	58.8	55.4	$p<0.001$
Experience from older children	47.6	43.1	35.9	42.7	NS
Child Health Centres personally	37.7	47.7	42.9	41.0	$p<0.05$
Relatives friends	38.5	33.8	44.1	39.9	NS
Child Health Centres pamphlets	30.7	41.5	31.8	32.6	NS
Magazines books	23.8	24.6	20.0	22.5	NS
Courses education	19.0	18.5	21.2	19.7	NS
Common sense	11.3	3.1	13.5	10.9	NS
Other sources	5.2	6.2	4.7	5.2	NS

in general was the same in the three groups although difficulties with the children's weight gain were experienced more frequently by the mixers ($p<0.01$) The mothers estimated knowledge and confidence in nutritional matters were the same for all three groups

Socio economic factors

The distribution of various socio economic factors with respect to the three feeding groups is shown in Table 5 The parents education and the mothers working seemed to have no influence on the choice of way of feeding and the amount of money spent on food did not differ between the groups (the family size did not differ either) All families had an extensive supply of helping devices although the jar buyers had more drying and ironing machines

Children of jar buyers were more often at home during the day ($p<0.001$) and more often always took their meals at home ($p<0.05$) The home cooking mothers often planned their shopping and did not buy on impulse ($p<0.01$) and furthermore they were doing more heavy household work e g baking ($p<0.001$) As a consequence perhaps they had not so much time for social contacts ($p<0.05$) but they were not so tired from their daily work as the jar buyers ($p<0.001$)

Sources of information

From Table 6 it is evident that information from the industries was the most important source for the parents knowledge about nutrition and feeding of babies irrespectively of their feeding practices This dominance was more marked among the jar buyers than in the two other groups ($p<0.001$) Then came experience from having older children and the information from Child Health Centres No difference regarding the sources of information was found with respect to the various age groups of children

DISCUSSION

It is easily conceivable that the rapid development of the baby food industry has influenced the feeding practices of children This is most apparent concerning breastmilk substitutes used for non breastfed infants in Sweden practically all are fed industrially produced infant formulas In the families studied about 80% used industrially produced Beikost—exclusively or in part—when foods other than milk were introduced (Table 1 age group 6–10 months) The age of introducing Beikost (=3–4 months) was later than in the United States where most infants receive such food by 6 weeks of age and many by 4 weeks of

age (2) However in toddlers and older infants home made foods accounted for a larger part of the Beikost as compared to younger infants thus in age group 12–18 months 61 2% got exclusively home made foods (Table 1) This is in agreement with the feeding practices in the United States (2)

Parents who preferred industrially produced Beikost were convinced that this was generally most adequate for the child although aspects of convenience also played an important role On the other hand the home cookers thought that their own table food was as adequate or nutritionally even better than that bought in jars It is interesting to note that 9 5% still believe that the industrially produced baby food contains harmful additives and preservatives in spite of heavy information campaigns by the industry on this matter

Estimates of intakes of calories and different nutrients were not made in this study but we have every reason to believe that no deficiency in these matters exists Studies on child health and nutrition in other areas both nearly and far away have all confirmed that the general health condition of children in Sweden is very good they are taller and heavier than children of corresponding age in other countries the haemoglobin concentration is generally satisfying only few have mild anemia and the nutritional requirements are well covered (5 6 7 8 9) *Feeding problems* still occur but do very seldom depend on lack of food Instead *they exist among* children who eat too little according to their parents opinion i e problems that are based on an emotional and behavioural level (4 10 11)

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The fact that social and economic factors to a very small extent seem to *influence the* choice of food for the children may imply that the economic situation among these families was so good that they could allow themselves a freedom of choice without deeper impact on their standard of living Instead it seems that the mother s own activities interest knowledge and ideas of how to feed children are decisive for her choice

So it is obvious that although the home cookers had a less complete supply of household machines they were more interested in household work like shopping baking and cooking and this did not exhaust them as much as it did the jar buyers

Information about baby food and nutrition is offered to parents from many quarters today both private and offcial ones Best resources in these matters are owned by the food industries Their information reach their customers by advertising and publicity campaigns in magazines and shops as well as by pamphlets sent directly to parents and furthermore also through professionals As a result information given by the food industries is the parents dominating source of knowledge about their children s nutrition and about feeding practices Even parents who preferred home made food used the food *industries* as their main source of information Under these circumstances it is of course most important that the information and the sales promotion activities by the baby food industries are of a *very high quality* and that they follow certain basic ethical principles These should protect the parents not only from getting false and biased facts but also against unduly persuasive advertising In a few countries e g in Sweden paediatricians as medical consultants to the industry have already formulated such *principles* (Medical Standards for Marketing of Infant Foods) which have been accepted by the industry (1) In other countries the development of such principles is in progress

Nevertheless information about nutrition and feeding practices should not be left solely to the industries no matter how good it is The primary responsibility for such information lies on the country s health authorities which consequently should make non commercial information material available The Child Health Centres have a traditional and

important role in advising parents in infant feeding Considering the apprehension of feed ing management often revealed by the mothers and also the relatively high frequency of feed ing problems reported it seems wise to educate and support the Child Health Centres towards a more active participation on nutri tional matters In this way they will perhaps reach a proper leading role as a source of knowledge for parents about the nutrition and feeding practices of their children

ACKNOWLEDGEMENT

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12 *Role of nutrition in public health* Report on a conference WHO Copenhagen 1977

Submitted Febr 17 1977
Accepted May 23 1977

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Acta Pædiatr Scand 66 673–675 1977

LINEAR GROWTH OF CHILDREN WITH LIMB DEFORMITIES FOLLOWING EXPOSURE TO THALIDOMIDE IN UTERO

C G D BROOK S N JARVIS and C G H NEWMAN

From the Departments of Paediatrics The Middlesex Hospital Wolfson Centre Institute of Child Health and Westminster Hospital London England

ABSTRACT Brook C G D Jarvis S N and Newman C G H (The Middlesex Hospital Wolfson Centre and Westminster Hospital London England Linear growth of children with limb deformities following exposure to thalidomide in utero Acta Paediatr Scand 66 673 1977 —The growth of 202 children exposed to thalidomide in utero and having upper (139 children) or lower (63 children) limb deformities has been assessed towards the end of pre pubertal growth The analyses show that children exposed to thalidomide are shorter than normal children but grow at a normal velocity later These findings may help in consideration of the mechanism by which thalidomide exerted its teratogenic effect Analyses of growth may find a wider use in the retrospective assessment of drugs which are potentially harmful in pregnancy

KEY WORDS Thalidomide environmental teratogens growth limb deformities

In 1961 attention was first drawn to the birth in the preceding two years of a number of children with various limb deformities which were attributed to the administration of thalidomide to mothers in early pregnancy (3) Despite extensive study the mechanism by which these occurred remains obscure (8) This paper reports the growth of some of these children in late childhood

MATERIALS AND METHODS

Cross sectional data were obtained from 0 children (115 boys 87 girls) aged 8 to 14 years 63 of them (36 boys 7 girls) had deformities of the lower limbs and the remainder of the upper limbs with normal lower limbs In the former sitting heights were recorded and in the latter heights were measured standing using standard anthropometric techniques (7)

In 10 boys and 1 girls longitudinal data on height were available between ages 8 and 1 3 of the boys and 1 of the girls were seen twice at annual intervals but the remainder were followed for 3 (3 boys girls) 4 (4 boys 6 girls) and 5 years (7 boys 1 girl) The measurements were plotted on standard charts and heights at annual intervals were taken from the straight lines connecting the points Height velocities were calculated in individual cases from the differences between the yearly estimates Since the children were of different ages the number of observations at any one age vary between 4 and 6

6 boys with lower limb deformities were followed longitudinally for a minimum of 2 years each and sitting height velocities were obtained in the same way

All data were compared to standard values (6) and the results are presented in terms of standard deviation scores (S D S) which were calculated in each case from the formula

$$\text{S D S} = \frac{x - \bar{X}}{s}$$

where x is the measurement $\bar{X}$ is the mean at that age and s the standard deviation

RESULTS

Table 1 shows the results of analysis of the cross sectional data All groups of children were significantly shorter than normal although the differences between the groups were not significant at the 5% level There remains nevertheless a suggestion that the growth of children with lower limb deformities was more severely affected than that of children with upper limb deformities

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Table 1 *Standard deviation scores of heights and sitting heights of children exposed to thalidomide in utero*

	No of children	Height or sitting height		χ^2	*P*
		Mean	S D		
Boys with upper limb deformity	69	−0 68	1 19	24 16	<0 001
Girls with upper limb deformity	48	−0 71	1 32	36 78	<0 001
Boys with lower limb deformity	36	−2 21	1 77	345 83	<0 001
Girls with lower limb deformity	27	−1 60	1 36	135 4	<0 001

children with upper limb deformities are shown in Table 2 These means correspond closely to the standard values at the various ages This indicates that the children although short are growing normally There were insufficient data to perform these calculations for the sitting height velocities of the children with lower limb deformities but the mean S D S of their sitting height velocities was +1 30 S D 1 28 which does not differ significantly from zero

DISCUSSION

The finding that children exposed to thalidomide in utero have a reduction in their growth potential is a new one It highlights the fact that the effects of thalidomide were not all or none This may have some bearing on the mechanism by which thalidomide caused its teratogenic effects which is not understood at all at the present time

Apart from the obvious limb deformities many body systems were affected including endodermal structures (intestinal atresias absence of appendix or gall bladder) mesodermal structures (skeleton heart kidneys genitalia) and ectodermal structures (cranial nerves eyes ears) Spinal changes have become clinically more apparent over the years and may influence stature (1) These changes included mild to moderate kyphoscoliosis abnormal epiphyseal plates or rarely fusion of *anterior vertebral bodies over several segments*

Spinal curvature of mild or moderate degree was noted in a few of our patients In the majority this seemed to be postural but X ray changes were found in 10 of the children with obvious deformities or symptoms in 5 of the

Table 2 *Height velocities of children exposed to thalidomide in utero*

Chronological age centre	No of observations	Height velocity of subjects		Standard values	
		Mean	S D	Mean	S D
Boys					
8 5	6	5 27	0 70	5 45	0 76
9 5	9	4 82	1 35	5 26	0 73
10 5	8	4 89	0 83	5 09	0 69
11 5	4	5 07	0 57	5 00	0 73
Girls					
8 5	5	5 24	0 37	5 51	0 79
9 5	6	6 17	0 72	5 47	0 81
10 5	10	5 95	0 68	5 73	0 92
11 5	7	6 44	1 67	7 41	1 06

Ref 5

group with lower limb deformities and in 5 of the group with upper limb deformities The heights of these children appeared randomly distributed in the groups as a whole We conclude therefore that whilst spinal changes may have contributed to short stature in a few children a more basic defect was responsible in the majority

The children of this series were apparently growing normally between the ages of 8 and 12 which rules out endocrine and other pathological causes for their short stature Nevertheless there was one child in this series (not included in the analysis) who had growth hormone deficiency which is now being treated (J M Tanner personal communication) The growth of the children is compatible with the hypothesis that thalidomide damaged the growth process at a time when subsequent catch up was not possible In this respect the children are similar to those who suffer prolonged intrauterine starvation in the second trimester and do not catch up (2) The later growth pattern of such children is identical to the presently reported one (5) The birth weights of the children in this series are not available but as standards for children missing limbs are not available either this point could not be resolved even if the data were there It is the clinical impression of the authors that the limb defects apart these children were distressingly normal as infants It seems unlikely that the growth findings are simply the result of low birth weight as in no other respect do the children resemble small for dates patients

The findings have implications for the assessment of teratogenicity of other substances administered to pregnant mothers At present the teratogenic effects of alcohol of smoking of anticoagulants and especially of anticonvulsants are under scrutiny (4) Growth analyses of the type reported in this paper may help in consideration of whether such substances are harmful to the fetus Measurements of growth are relatively easily performed and have the advantage of being able to be applied retrospectively

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Acta Pædiatr Scand 66 677–680 1977

THE EARLY DETECTION OF PNEUMOTHORAX WITH TRANSTHORACIC IMPEDANCE IN NEWBORN INFANTS

G NOACK and U FREYSCHUSS

From the Department of Pediatrics Karolinska Institutet at S t Goran s Children s Hospital and the Department of Clinical Physiology Serafimer Hospital Stockholm Sweden

ABSTRACT Noack G and Freyschuss U (The Department of Pediatrics Karolinska Institutet at S t Goran s Children s Hospital and the Department of Clinical Physiology Serafimer Hospital Stockholm Sweden) The early detection of pneumothorax with trans thoracic impedance in newborn infants Acta Paediatr Scand 66 677 1977 —During the treatment of IRDS with assisted ventilation pneumothorax is a common and dangerous com plication where an early diagnosis is important for the successful treatment In this case report the continuous monitoring of transthoracic electrical impedance has proven to be a sensitive and non invasive method to detect the development of pneumothorax long before clinical signs are manifest

KEY WORDS pneumothorax newborn transthoracic electrical impedance

Assisted ventilation is indispensible in the treatment of the idiopathic respiratory distress syndrome of the newborn and especially the introduction of continuous positive airway pressure (CPAP) by Gregory et al (6) has added greatly to the ventilatory efficiency Unfortunately neither prolonged ventilatory assistance nor CPAP with spontaneous ven tilation are without complications Pneumothorax and pneumomediastinum occuring with or without mechanical ventilation are one of the leading immediate causes of mortality (6) The need for an early diagnosis of pulmonary complications is thus urgent This report shows that the measurement of transthoracic electrical impedance is a valuable technique for the early detection of pneumothorax

METHODS

Transthoracic electrical impedance was measured by a Minnesota Impedance Cardiograph Model 304 A A tetra polar electrode system was used aluminium electrodes with mylar adhesive backing were placed circumferenti ally around the patient s neck and chin and at the level of the diaphragm The outer electrodes 1 and 4 were con nected to a constant current source providing 4 mA at a *frequency of 100 kHz* From the two inner electrodes 2 and 3 the standing impedance i e basal impedance (Z_0) was read on a digital display and the first deriva tive of the diminution of Z_0 occurring during the cardiac *cycle* (dZ/dt) was displayed on a recorder A crystal micro phone was placed at the second left intercostal space to record a phonocardiogram thus facilitating a proper tim ing of the 2nd heart sound The electrodes radiolucent to X ray could be kept in place for as long as 10 to 14 days

Stroke volume was calculated according *to a modifi* a tion of the method of Kubicek et al (7) utilizing the follow ing formula

$$\text{Stroke volume} = \rho \left(\frac{L}{Z}\right)^2 T \; dZ/dt \; \text{min}$$

where

- ρ = resistivity of blood (determined from the hemato crit)
- L = distance (cm) between the inner electrodes 2 and 3
- Z_0 = the basal thoracic impedance (ohm)
- T = the ventricular ejection time (sec)
- dZ/dt = the maximum deflection of impedance trace *from* zero line (ohm/sec)

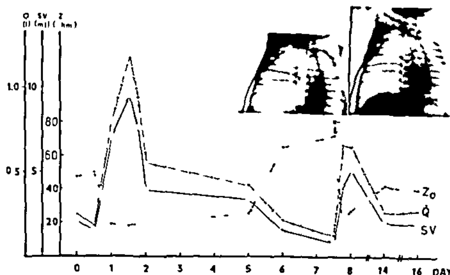


Fig 1 Consecutive transthoracic impedance data of case 1 $\dot{Q}$=cardiac output SV=cardiac stroke volume Z_0=basal impedance X ray pictures demonstrate pneumothorax and the effect of subsequent pleural suction

On a multichannel graphic recorder (Siemens Elema Mingograph 34) three output signals were recorded ECG dZ/dt and phonocardiogram As baseline shifted with respiration only tracings at the zero line were selected for calculation i e the same procedure as during recording in apnoea

Arterial blood gases (P_{aO_2} P_{aCO_2}) and pH were analyzed with a blood micro system (BMS2 Radiometer Copenhagen)

CASE REPORT

Case 1 Twin no 1 a premature boy weighing 1 340 g and with Apgar 4 breathed spontaneously after external stimulation He was brought to the intensive care unit with repeated episodes of apnoea A catheter was inserted into the abdominal aorta through an umbilical artery He was treated with external stimulation and 80% oxygen but cyanosis and bradycardia reappeared CPAP was started but a P_{aO_2} of 6 6 kPa (50 mmHg) and a base excess of 11 mmol/l were an indication for endotracheal intubation and assisted ventilation with respirator (Servo Ventilator Siemens Elema) with IPPB (intermittent positive pressure breathing) and PEEP (positive end expiratory pressure) of +4 cmH O

X ray film showed atelectases in both lungs associated with a low basal impedance a large stroke volume and cardiac output (Fig 1) which might reflect an increased shunt After

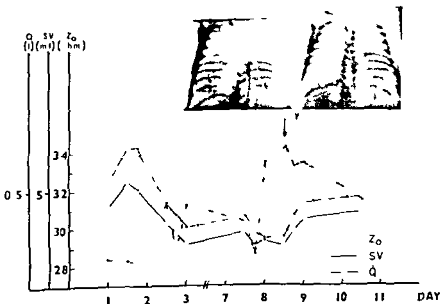


Fig 2 Sequence of impedance data and X rays of case 2 Abbreviations as in Fig 1

mechanical ventilation for four days the basal impedance slowly increased and the stroke volume and cardiac output decreased The blood gases were normal with a P_{ao} of 11 6 kPa (87 mmHg) and a pH of 7 34 Between the 5th and the 6th day of birth the impedance rose to over 60 ohm without clinical signs of distress In the middle of the 7th day the infant became cyanotic and blood pressure fell X ray then showed a right pneumothorax with dislocation of the heart (Fig 1) Stroke volume and cardiac output were under these circumstances low With 90% oxygen in the inspiratory gas P_{O_2} was 10 1 kPa (75 mmHg) and pH 7 45 After thoracocentesis and permanent suction the infant recovered and impedance data normalized

Case 2 A newborn boy birth weight 1765 g was delivered by caesarean section Due to apnoea endotracheal intubation and ventilation with respirator was commenced at the intensive care unit Admission Z_0 was 28 4 ohm and evidence of bilateral infiltration was noted on the X ray P_{O_2} was 8 5 kPa (64 mmHg) During the following days with mechanical ventilation and PEEP+4 cmH_2O Z_0 rose to about 30 ohm and chest film was clearer The basal impedance remained at this level until the 7th day after birth when Z_0 rose from 28 6 to 34 4 ohm X ray demonstrated a partial pneumothorax but clinical signs were absent

On the 8th day the infant developed cyanosis and bradycardia X ray showed pneumothorax at the right side and P_{aO_2} was 6 6 kPa (50 mmHg) Z_0 dropped after thoracocentesis and permanent suction The hospital course is illustrated in Fig 2 The infant later redeveloped pneumothorax and died

DISCUSSION

The use of transthoracic impedance as a reliable and sensitive non invasive measure of air fluid changes in thorax is well documented in both experimental and clinical studies (1–4 9–13) Pulmonary infiltrate and pleural effusion decrease basal impedance positive pressure ventilation and pneumothorax increase impedance The impedance variations during respiration has been used for apnoea monitoring (3) The sensitivity of the method to detect alterations in intrathoracic fluid volumes has been emphasized by Pomerantz et al (11) who observed a fall in basal impedance 45 min before other parameters such as blood gases central venous pressure and pulmonary compliance significantly changed

For newborn infants with pulmonary complications it is important to have a continuous monitor of a non invasive type which does not disturb their normothermic atmosphere and which does not interfere with therapy or other diagnostic interventions e g X ray

The present report demonstrates the pitfall of the early phase of a developing pneumothorax where obvious clinical signs are absent and hence therapy might be delayed Several X rays of the chest are needed because of the initial pulmonary dysfunction the number of radiation exposures will greatly increase if secondary pulmonary complications are added Thus X ray was performed twelve times in case 1

In our two cases a rapid significant increase in basal impedance was associated with pneumothorax and falling impedance values with pleural suction The accuracy of the stroke volume – cardiac output data in the present study cannot be validated It can only be assumed that the initial large stroke volume noted in case no 1 might reflect an increased shunt in accordance with the observation that in patients with left to right shunts the impedance cardiac output values correlated well with pulmonary blood flow (8)

Normal values of basal impedance are not available due to its dependance on e g thoracic volume In an ongoing study healthy neonates are followed after vaginal delivery and caesarian section respectively (5) During the first 36 hours the mean intraindividual changes in basal impedance were 2 1 (S D ±

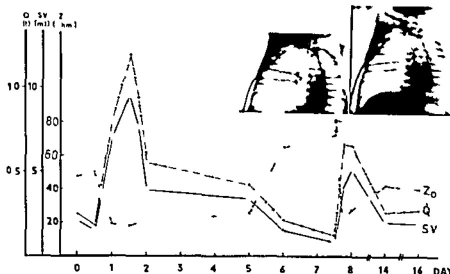


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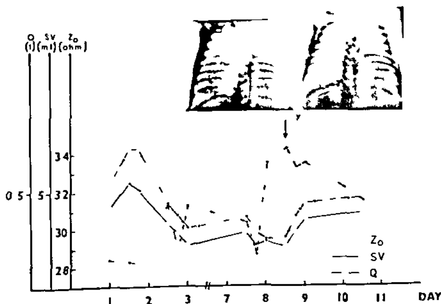


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Acta Pædiatr Scand 66 681–687 1977

SERUM LEVELS OF THYROTROPIN THYROXINE AND TRIIODOTHYRONINE IN FULLTERM SMALL FOR GESTATIONAL AGE AND PRETERM NEWBORN BABIES

B BROCK JACOBSEN H J ANDERSEN B PEITERSEN H DIGE PETERSEN and L HUMMER

From the University Clinic of Paediatrics Children s Hospital Fuglebakken and from the Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark

ABSTRACT Jacobsen B B Andersen H J Peitersen B Dige Petersen H and Hummer L (The University Clinic of Paediatrics Children s Hospital Fuglebakken and the Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark) Serum levels of thyrotropin thyroxine and triiodothyronine in fullterm small for gestational age and preterm newborn babies Acta Paediatr Scand 66 681 1977.—Simultaneous serum concentrations of TSH total thyroxine (T_4) and triiodothyronine (T_3) were determined in 93 fullterm (FT) 37 small for gestational age (SGA) and 38 preterm (PT) babies with a post natal age from 2 to 144 hours In addition TSH T_4 and T_3 concentrations were measured in cord sera from 27 FT 4 SGA and 5 PT babies and in venous blood from 20 mothers at delivery Cord blood concentrations of TSH were higher and T_4 and T_3 concentrations were lower than seen in the mothers Serum concentrations of TSH were high during the first day of life followed by a decline There was no statistically significant difference between serum TSH concentrations of the three groups of newborns On the 5th day of life no elevated serum TSH values were found in any of the groups (TSH<5mU/l) Serum concentrations of thyroid hormones increased after birth and reached maximum levels within 24 hours in all groups The relative increases above cord level were of the same magnitude in the newborns Two times for serum T and six times for serum T_3. The thyroid hormone concentrations in blood samples from FT babies decreased from the second day of life whereas in low birth weight newborns the decreases were more variable The serum levels of T and T_3 were significantly different in the three groups of newborns the highest values were seen in FT and the lowest values in PT babies In contrast the ratios between molar serum concentrations of T and T were found to be highest in PT lower in SGA and lowest in FT babies approaching maternal values during the first week of life The data are discussed with regard to hormone secretion thyroxine binding capacity and peripheral T to T_3 conversion in the three groups of newborns It is concluded that from day 5 after birth serum TSH determinations alone or in combination with serum T_4, seem to be the method of choice in screening for congenital hypothyroidism

KEY WORDS Thyrotropin thyroxine triiodothyronine preterm small for gestational age fullterm newborns

In fullterm newborn babies the postnatal hypersecretion of thyrotropin (TSH) has been described by several authors (7 12 13 26) the subsequent increases in serum concentrations of protein bound iodine (PBI) thyroxine (T_4) and triiodothyronine (T_3) are also well known (1 8 11 29 32) In lowbirth weight neonates however simultaneous values of serum TSH T_4 and T_3 have not been published Because of low serum T_4 concentrations in preterm and small for gestational age babies (23 33) these newborns may be falsely suspected of neonatal hypothyroidism

The clinical diagnosis of hypothyroidism during the neonatal period is difficult and often overlooked (3) and neonatal screening pro-

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Transthoracic impedance techniques have been used for apnoea monitoring in neonatal intensive care units Due to variations in apparatus many of which are home made and due to different electrode techniques data on basal impedance if available cannot be extrapolated to other equipment To our knowledge an evaluation of basal impedance changes obtained with a commercially available apparatus as done above against a healthy control material has not been reported A method is hereby offered for the detection of changes in the respiratory state of an infant long before they are clinically significant

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Submitted Dec 23 1976
Accepted May 23 1977

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Acta Pædiatr Scand 66 681–687 1977

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Table 2 *Median and range of serum TSH concentration (mU/l) in newborn babies*
Maternal serum TSH concentrations at delivery ranged 0 6–4 0 (median 1 9) mU/l *n* = number of subjects

	Cord blood	Postnatal days 1		2	3	4	5	6
		0-1 h	13–24 h					
FT								
Median	3 4	23 6	14 8	5 4	2 3	2 3	1 8	1 2
Range	0 8–14 4	10 8–120	3 0–33	2 7–30	0 3–8 3	0 6–3 7	0 3–3 2	<0 1–2 6
(*n*)	(5)	(8)	(7)	(15)	(14)	(11)	(14)	(12)
SGA								
Median	16 9	3 2	12 7	11 8	4 0	2 6	2 5	0 8
Range	9 3–16 9	0 7–58	3 4–21 9	2 8–22 4	2 7–13 1	0 1–6 3	1 4–2 8	0 4–1 6
(*n*)	(3)	(3)	(2)	(7)	(7)	(4)	(3)	(3)
PT								
Median	9 2	9 4	7 2	3 9	5 0	3 3	3 6	2 0
Range	1 8–15 8	4 9–38	0 1–72	1 0–17 0	1 6–13 1	0 3–6 3	<0 1–5 0	0 3–3 7
(*n*)	(5)	(3)	(5)	(9)	(5)	(2)	(6)	(2)

above this range The serum TSH levels in PT and SGA neonates were however so highly variable that no statistically significant decrease could be shown after 24 hours After the 5th day of life all values were below 5 mU/l

In cord sera the TSH concentrations of PT and SGA babies seemed to be higher than in FT but few low birth weight babies were studied Maternal TSH values were within the normal range

Serum T_4 concentrations (Table 3)

In the FT newborns maximum values of serum T_4 were found about 24 hours after delivery and they were about twice those of cord blood (Fig 1) After this time a significant decrease in serum T_4 concentrations appeared ($p<0\ 01$) The maximum serum T_4 values of PT and SGA newborns were about twice the cord levels as in FT babies The serum T_4 concentrations in the 3 groups of newborns differed significantly ($p<0\ 001$) the lowest

Table 3 *Median and range of serum T_4 concentrations (nmol/l) in newborn babies*
Maternal serum T concentrations at delivery ranged 116–270 (median 175) nmol/l *n* = number of subjects

	Cord blood	Postnatal days 1		2	3	4	5	6
		0–12 h	13–24 h					
FT								
Median	13	37	296	253	267	238	216	224
Range	103–206	178– 83	231–330	196–3 7	136–430	139–437	157–314	154– 65
(*n*)	(27)	(9)	(9)	(16)	(18)	(15)	(13)	(13)
SGA								
Median	1 1	18	60	2 5	242	206	24	2 6
Range	90–1 7	118–290	139–296	144–314	1 6–291	147– 80	142–320	154–304
(*n*)	(4)	(5)	(5)	(10)	(5)	(5)	(3)	(4)
PT								
Median	93	161	180	177	156	155	157	160
Range	87–144	118–185	129–258	85– 88	99–190	54–189	142–2 7	160–160
(*n*)	(5)	(4)	(7)	(8)	(6)	(4)	(7)	(2)

Table 1 *Clinical data of fullterm (FT) small for gestational (SGA) and preterm (PT) newborn babies*

	No of infants	Sex		Gestational age (w)	Birth weight (g)	Postnatal age (h)
		♀	♂			
Cord						
FT	27	10	17	39 (39–40)	3 500 (2 850–4 250)	–
SGA	4	2	2	39 (37–42)	2 650 (1 800–2 900)	–
PT	5	1	4	34 (25–36)	2 600 (1 000–2 800)	–
Peripheral vein						
FT	93	47	46	39 (37–41)	3 450 (2 850–4 500)	62 (4–144)
SGA	37	17	20	39 (37–40)	2 450 (1 790–2 900)	48 (4–144)
PT	38	20	18	35 (27–36)	2 140 (850–2 750)	46 (2–129)

Values shown are median and range

cedures for congenital hypothyroidism will therefore be of increasing importance (2 16)

The present study therefore was undertaken to investigate the changes in simultaneous serum levels of TSH T_4 and T_3 in normal preterm and small for gestational age newborns compared to fullterm newborn babies during the first six days of life

MATERIALS AND METHODS

The clinical data are presented in Table 1 Venous blood samples were obtained from 93 fullterm (FT) babies 37 small for gestational age (SGA) babies with gestational age from 37 to 40 weeks and from 38 preterm (PT) appropriate for weight babies with gestational age from 27–36 weeks The parents were informed and consent obtained The birth weights of the three groups differed significantly ($p<0\ 001$) Gestational age and maturity were assessed as previously reported (19 20) The postnatal age ranged from 2 to 144 hours median values tended to be lower in low birth weight newborns but did not differ significantly from that of FT babies ($p>0\ 05$) In addition cord blood samples were obtained from 27 FT 4 SGA and 5 PT babies Infants with a history of fetal distress respiratory distress syndrome or major congenital abnormalities were excluded from the study Finally venous blood samples were obtained from 20 mothers at the delivery The infants of these mothers were FT and included in the present study

Only one blood sample was obtained from each infant Unfortunately in some cases there was only material for measurements of two of the hormones one of which was T_4 Blood was usually drawn in the morning centrifuged immediately and serum was stored at −20° until analyses were performed *Serum TSH* was measured by double antibody RIA technique as previously reported (10 20) The detection limit of the assay is 0 2 mU/l and in the calculations values below the sensitivity limit were expressed as 0 1 mU/l The serum TSH concentration in adults is <3 5 mU/l *Serum T_4* was determined using a modification of the competitive binding technique (Tetralute Ames) reported by Braverman et al (4) and elsewhere by us (20) Normal adult range of serum T_4 concentration is 60–137 nmol/l *Serum T_3* was measured radioimmunologically (17 20) Normal adult range of serum T_3 concentration is 1 10–2 40 nmol/l All analyses were performed in duplicate

A normal distribution of the thyroid variables was not seen A non steady state of hormone secretion during the first days of life and the differences (for obvious reasons) with regard to postnatal age and number of infants in the groups of newborns make a comparison between the hormone levels difficult Very low p values however support the assumption that the null hypothesis" (31) (no true differences between the groups) must be discharged The following non parametric statistical tests were used The Mann Whitney test the Kruskal Wallis one way analysis of variance and the Spearman rank correlation (R) (9 31)

RESULTS

Serum TSH concentration (Table 2)

In all the FT babies maximum values of serum TSH were seen within 24 hours after birth and subsequently a significant decrease with postnatal age was observed ($p<0\ 0005$) normal adult levels were reached from 72 to 96 hours after delivery In low birth weight newborns high serum TSH concentrations were observed within the first 24 hours of life as in FT babies and the TSH values were not significantly different from those of FT ($p\geq 0\ 2$) a few TSH values below the range of FT were observed none had a serum TSH (0–24 hours)

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Median	9 ?	9 4	7 2	3 9	5 0	3 3	3 6	2 0
Range	1 8–15 8	4 9–38	0 1–7?	1 0–17 0	1 6–13 1	0 3–6 3	<0 1–5 0	0 3–3 7
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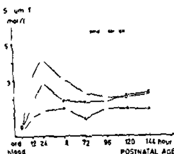


Fig 2 Median values of serum T_3 concentrations during the first 6 days of life in normal fullterm (*FT*) small for date (*SGA*) and preterm (*PT*) babies

serum concentrations of T_4 and T_3 were calculated Median values and range of the T_4/T_3 ratios are shown (Fig 3) The ratio was higher in cord blood than in bloodsamples from day 1 to 6 of life and much higher than observed in maternal blood samples The T_4/T_3 ratio in PT was significantly higher than in SGA babies and their ratios were higher than seen in FT babies ($p<0\ 0005$) The T_4/T_3 ratio decreased by age and approached that of maternal values

DISCUSSION

The present study demonstrates pronounced changes in serum concentrations of TSH and thyroid hormones in FT as well as in SGA and PT newborn babies

In FT babies there was invariably a pronounced early increase and a subsequent decrease in serum TSH values reaching normal adult levels after 2–3 days cord blood levels of TSH were higher than maternal values This agrees with previous reports (7 12 13 14 32) Our finding of a higher TSH level in cord blood of low birth weight babies than seen in FT babies is not in accordance with a previous study by Fisher et al (14) and might be due to the rather small number of cord blood samples from SGA and PT babies in the present study

In low birth weight neonates the serum TSH concentrations did not differ significantly from that of FT babies but the changes in serum TSH in relation to time were more variable compared with the FT neonates After 5 days no elevated serum TSH concentrations were found in any groups This does not seem to be in keeping with the findings of Lemarchand Beraud et al (24) describing a more marked and sustained TSH release in PT (and some SGA) babies with elevated serum TSH concentrations even after one week However the TSH analysis of these authors differs from ours by use of canine serum instead of TSH free human serum for the standard curve Furthermore nothing was reported concerning body temperature All our infants had normal body temperature and many of the low birth weight newborns were in incubator at the time of study The influence of cold (12) could be one cause of the difference between the two materials

The present data of TSH values in PT babies *during the first week of life* and the normal TSH response to exogenous thyrotropinreleasing hormone (TRH) in PT (and SGA) neonates (20) support the hypothesis of a maturation of the fetal hypothalamicpituitary system prior to the 27th week of gestation (14 18)

The serum concentrations of thyroid

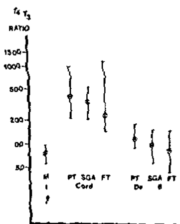


Fig 3 Median values and range of the ratios between molar serum concentrations of T_4 and T_3 in fullterm (*FT*) small for gestational age (*SGA*) and preterm (*PT*) new born babies and in the mothers at delivery (*Cord* cord sera *day 1–6* serum samples from babies 2 to 144 hours of age)

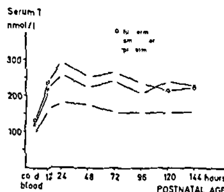


Fig 1 Median values of serum T_4 concentrations during the first 6 days of life in normal fullterm (*FT*) small for date (*SGA*) and preterm (*PT*) babies

values were observed in PT No statistically significant decrease after 24 hours could be shown in PT and SGA babies In all groups the median T_4 level remained above normal adult level throughout the period of study

In cord sera the serum T_4 concentrations of all newborns were significantly lower than maternal values ($p<0\ 001$) and seemed to be lower in the low birth weight newborns than in FT

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In cord blood the same distribution between the groups was observed as for serum T_4 PT babies seemed to have the lowest values

Relationship between serum concentrations of T_4 and T_3

In cord blood of FT babies the higher T_4 levels correlated with the higher T_3 levels ($R=0\ 54$ $p<0\ 01$) and this seemed also to be the case in PT and SGA babies During the neonatal period (day 1 to 6) a positive correlation was found in all groups ($R=0\ 59$ $0\ 79$ and $0\ 82$ in FT SGA and PT babies respectively $p<0\ 001$)

In all subjects with measurement of both thyroid hormones the ratios between molar

Table 4 *Median and range of serum T_3 concentrations (nmol/l) in newborn babies*
Maternal serum T_3 concentrations at delivery ranged 1 70–2 77 (median 2 71) nmol/l n=number of subjects

	Cord blood	Postnatal days 1		2	3	4	5	6
		0–12 h	13–24 h					
FT								
Median	0 53	3 10	4 21	3 11	2 33	2 07	2 03	2 28
Range	0 11–0 96	2 97–6 26	3 04–6 00	1 44–5 29	0 86–5 31	1 66–4 71	1 63–3 72	1 52–4 46
(n)	(26)	(8)	(8)	(15)	(16)	(12)	(14)	(12)
SGA								
Median	0 47	1 62	2 91	1 96	1 82	1 88	2 15	2 27
Range	0 35–0 60	1 62	1 66–4 90	0 89–2 33	1 00–2 35	1 40–2 76	2 02–2 73	1 96–3 22
(n)	(2)	(1)	(3)	(5)	(4)	(6)	(4)	(3)
PT								
Median	0 20	1 00	1 34	1 53	0 89	1 46	1 45	1 35
Range	0 09–0 65	0 77–1 22	0 86–1 57	0 86–2 05	0 78–1 28	1 46	0 83–1 66	1 30–1 40
(n)	(5)	(2)	(5)	(7)	(6)	(1)	(4)	(2)

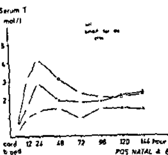


Fig 2 Median values of serum T_3 concentrations during the first 6 days of life in normal fullterm (*FT*) small for date (*SGA*) and preterm (*PT*) babies

serum concentrations of T_4 and T_3 were calculated Median values and range of the T_4/T_3 ratios are shown (Fig 3) The ratio was higher in cord blood than in bloodsamples from day 1 to 6 of life and much higher than observed in maternal blood samples The T_4/T_3 ratio in PT was significantly higher than in SGA babies and their ratios were higher than seen in FT babies ($p<0.0005$) The T_4/T_3 ratio decreased by age and approached that of maternal values

DISCUSSION

The present study demonstrates pronounced changes in serum concentrations of TSH and thyroid hormones in FT as well as in SGA and PT newborn babies

In FT babies there was invariably a pronounced early increase and a subsequent decrease in serum TSH values reaching normal adult levels after 2–3 days cord blood levels of TSH were higher than maternal values This agrees with previous reports (7 12 13 14 32) Our finding of a higher TSH level in cord blood of low birth weight babies than seen in FT babies is not in accordance with a previous study by Fisher et al (14) and might be due to the rather small number of cord blood samples from SGA and PT babies in the present study

In low birth weight neonates the serum TSH concentrations did not differ significantly from that of FT babies but the changes in serum TSH in relation to time were more variable compared with the FT neonates After 5 days no elevated serum TSH concentrations were found in any groups This does not seem to be in keeping with the findings of Lemarchand Béraud et al (24) describing a more marked and sustained TSH release in PT (and some SGA) babies with elevated serum TSH concentrations even after one week However the TSH analysis of these authors differs from ours by use of canine serum instead of TSH free human serum for the standard curve Furthermore nothing was reported concerning body temperature All our infants had normal body temperature and many of the low birth weight newborns were in incubator at the time of study The influence of cold (12) could be one cause of the difference between the two materials

The present data of TSH values in PT babies during the first week of life and the normal TSH response to exogenous thyrotropinreleasing hormone (TRH) in PT (and SGA) neonates (20) support the hypothesis of a maturation of the fetal hypothalamic-pituitary system prior to the 27th week of gestation (14 18)

The serum concentrations of thyroid

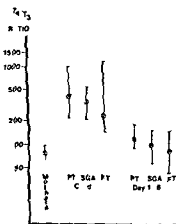


Fig 3 Median values and range of the ratios between molar serum concentrations of T_4 and T_3 in fullterm (*FT*) small for gestational age (*SGA*) and preterm (*PT*) newborn babies and in the mothers at delivery (*Cord* cord sera *day 1–6* serum samples from babies ? to 144 hours of age)

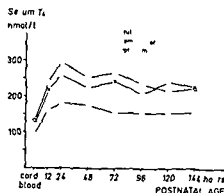


Fig 1 Median values of serum T_4 concentrations during the first 6 days of life in normal fullterm (*FT*) small for date (*SGA*) and preterm (*PT*) babies

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(n)	(5)	(2)	(5)	(7)	(6)	(1)	(4)	(2)

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Submitted Febr 18 1977
Accepted May ?3 1977

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hormones were low in cord sera and increased during the first 24 hours after birth Previously similar findings have been reported in FT babies (1 7 11 25 26 32) The relative serum T_3 increase was more marked than the serum T_4 increase corresponding to the higher relative T_3 response in newborns after exogenous TRH stimulation (20)

The total serum concentrations of T_4 and T_3 during this first week of life were highest in FT and lowest in PT babies whereas intermediate values appeared in SGA babies Previously lower serum PBI and T_4 concentrations have been observed in some low birth weight newborns (21 27 30) and some studies have demonstrated that the total and free serum T_4 and thyroxinebinding globulin (TBG) concentrations increase progressively with gestational age (14 18) Therefore the low values found in PT in the present study may be due to low serum concentrations of TBG this may be the reason for the lower thyroid hormone values in SGA babies too Further studies are in progress

In all groups of newborns a positive correlation was found between serum concentrations of T_4 and T_3 However not only the serum levels were different but also the *ratios* between serum T_4 and serum T_3 differed in FT SGA and PT newborns Previous studies of FT infants have demonstrated a high ratio of serum T_4 to serum T_3 concentrations in cord blood as compared with maternal values and a pronounced decrease after delivery (1 11) In cord blood of PT babies even higher T_4/T_3 ratios have been observed although in the fetal thyroid gland the ratio of T_4/T_3 after midgestation is similar to that of adults (15) These observations are in accordance with the results presented The findings agree with the suggestion of a relatively low rate of T_4 to T_3 conversion by extra thyroidal tissue in the fetus as compared to the newborn child (1 11 15 22) When calculating the serum T_4/T_3 ratio of the neonates during the first 6 days of life we found that the ratios differed significantly in the 3 groups with the highest values in PT and lowest values in FT babies This may indicate that a relative T_3 deficiency to some extent continues in low birth weight neonates during the early postnatal period Chopra et al (5) have recently reported high concentrations of reverse triiodothyronine in cord blood and a pronounced decline in FT babies after birth Investigations of the relationship between the serum values of T_4 T_3 and reverse T_3 in the different groups of newborns would be of great interest In addition the reports of a lower serum T_4 and serum T_3 and a higher ratio of T_4/T_3 in PT babies with respiratory distress syndrome than in controls attract attention but require further investigations (6 28)

This study shows that in low birth weight newborns the measurement of serum T_4 alone may falsely indicate primary hypothyroidism After 5 days of age the serum TSH concentration was normal in all newborns including PT and SGA babies We therefore conclude that a supplementary measurement of TSH should be performed from the 5th day of life to exclude the diagnosis of congenital hypothyroidism

ACKNOWLEDGEMENTS

We are grateful for excellent technical assistance by the staff of the two laboratories involved in the project and for valuable secretary help by Miss A Jacobsen

Human TSH for iodination and anti human TSH were kindly donated by the National Pituitary Agency Bethesda USA The human TSH international reference preparation 68/38 was given by the Medical Research Council Holly Hill London Thanks also to the Department of Obstetrics Frederiksberg Hospital from which some of the blood samples were obtained

The study was supported by Statens Laegevidenskabelige Forskningsfond and Dagmar Marshall Foundation

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Acta Pædiatr Scand 66 689–692 1977

ANTIBODY RESPONSE IN MARASMIC CHILDREN DURING RECOVERY

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From the Nutrition Research Program and the Department of Clinical Pathology Hospital of the American University of Beirut Beirut Lebanon

ABSTRACT Awdeh Z L, Kanawati A K and Alami S Y (The Nutrition Research Program and the Department of Clinical Pathology Hospital of the American University of Beirut Beirut Lebanon) Antibody response in marasmic children during recovery Acta Paediatr Scand 66 689 1977 —The I II and III antibody responses to tetanus and diphtheria were assessed in marasmic and moderate protein-calorie malnourished children and compared to that of controls The results suggest that during recovery marasmic children are capable of responding adequately to tetanus and diphtheria vaccines

KEY WORDS Antibody response marasmus

Malnutrition is thought to increase the susceptibility of children to infection and the consequences of infectious diseases are likely to be more serious In children suffering from kwashiorkor there is evidence of increased frequency and severity of infections (11) There is also good evidence that both the cellular (14 15) and humoral (9) immunity are depressed in such children The effects of marasmus on immunity in such children is either decreased (12) unchanged or increased (4) The results of studies on the humoral response are also conflicting (1 7)

Marasmus is the most common form of malnutrition in many developing countries (6) it mainly affects infants between the age of 3–12 months The weight of the child suffering from marasmus may fall below 50% of his expected weight for age Treatment requires hospitalization for about three months during which time the child may approach his expected weight for age

In view of the fact that little is known about the effectiveness of vaccines given to marasmic children we decided to assess their immunological response to the triple vaccine by measuring the level of antitetanus and antidiphtheria antibodies in their serum after vaccination On the same samples obtained for the measurement of the levels of antibodies quantitation of IgG IgA and IgM immunoglobulins was performed to determine if they are low in marasmus and if they vary during recovery

MATERIALS AND METHODS

Subjects

The study was conducted on three groups of children between the age of 3–12 months The first group consisted of marasmic children with body weight less than 60% of Boston International Standard The children in this group had marked wasting of subcutaneous tissues with no oedema or any of the other signs of Kwashiorkor They were studied two weeks after admission to the hospital and after the treatment of diarrhea vomiting and electrolytes imbalance which were frequently present

The second group consisted of children admitted to the hospital with moderate protein-calorie malnutrition (PCM) They were non-oedematous with body weight

Table 2 *Mean immunoglobulin levels in malnourished children before and after treatment as compared to controls*

		No of children	% weight for age	Level (g/litre)		
				IgG	IgA	IgM
Marasmus	Before recovery	10	55 0± 4 4	5 75±2 47	0 34±0 23	1 61±0 42
	After recovery	10	77 1± 4 4	7 33±3 43	0 28±0 15	1 52±0 82
Moderate PCM	Before recovery	11	75 1± 5 9	6 52±2 52	0 29±0 19	1 17±0 55
	After recovery	11	88 1±10 9	8 67±3 44	0 24±0 10	1 07±0 35
Control		35	103 0± 7 0	5 69±2 80	0 25±0 19	0 98±0 50

erately malnourished group was not significantly different ($p>0$ 05)

The mean levels of IgG IgA and IgM immunoglobulins are given in Table 2 Since immunoglobulins levels in infants are age dependent the statistical analysis was done by the analysis of covariance (16) There were no significant differences ($p>0$ 05) between the control the marasmic and the moderately malnourished groups for the three immunoglobulin classes IgG IgA and IgM at the start of the treatment or after recovery Furthermore there was no significant difference ($p>0$ 25) between any of the three immunoglobulin levels at the start of treatment and after recovery

DISCUSSION

It has been suggested that the most important two factors that determine the well being of infants in any community are nutritional status and freedom from disease A combination of malnutrition and infections is responsible for the high infant mortality rate in developing countries (8 13) In communities where malnutrition is common there is no clear recommendation as to whether children suffering from malnutrition should be vaccinated In this country and perhaps in other developing countries there is an impression that vaccinating a malnourished child may not be advisable and that it may even be harmful since the response of such children may not be adequate In this respect there is nothing in our data to suggest that marasmic children should not be vaccinated with the triple vaccine since such children are capable of responding normally to this vaccine provided that they are being treated for malnutrition

ACKNOWLEDGEMENT

This work was supported by a grant from WHO and in part by a grant from the Lebanese National Council for Scientific Research

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Table 1 *Mean tetanus and diphtheria titers for malnourished and normal children*
All initial blood samples had no detectable antitetanus or antidiphtheria antibodies

Group	Response	No of children	% weight for age	Mean tetanus titre $\log_2$	Mean diphtheria titre $\log_2$
Normal	Primary	6	91 3±13 5	3 9± 1 2	3 3±0
	Secondary	6	92 9±12 4	7 5± 4 1	4 6±2 1
	Tertiary	6	93 5±12 0	6 9± 3 5	3 9±0 5
Moderate PCM	Primary	11	75 1± 5 9	5 4± 2 7 NS	4 9±2 4 NS
	Secondary	11	79 8±10	7 6± 2 9 NS	6 5±0 3 NS
	Tertiary	10	84 9± 9 7	9 5± 2 8 NS	7 3±0 8 S
Marasmus	Primary	9	55 0± 4 4	6 0± 3 3 NS	6 3±3 4 NS
	Secondary	10	66 0± 7 2	9 3± 1 4 NS	7 3±1 8 NS
	Tertiary	6	73 8± 7 4	10 2±10 5 S	8 6±1 9 S

NS and S indicate the result of *t* test as compared with the response of the control group NS=not significant ($p>0$ 05)
S=the difference was significant ($p<0$ 05)

between 60–80% of Boston International Standard The malnourished children in both groups were fed S26 humanized milk (Wyeth Laboratories Inc Philadelphia Pa) and mixtures of Laubinia which is a protein enriched cereal food (2) cow s milk and sugar The third group consisted of normal healthy children with a body weight above 90% of the Boston International Standard the children came from the same environment to act as age matched controls There was no control on the diet of children in the control group as they were not hospitalized The weights of the children in three groups were expressed as a percentage of their expected standard weight for age at the start of vaccination using the Boston International Standard as a reference

An initial blood sample was obtained from all children of all groups before receiving the first dose of the triple vaccine Two weeks later a second blood sample was obtained At two weeks interval blood sample drawing was alternated with revaccination until a total of three inoculations (i e given at monthly intervals) had been made and four blood samples obtained On each serum sample obtained the levels of antidiphtheria and antitetanus antibodies as well as that of the IgG IgA and IgM immunoglobulins were determined Since the levels of immunoglobulins in children are age dependent and since the number of children who received the vaccination in the control group was small immunoglobulin levels of 35 children attending the well baby clinic were also determined

Vaccines

Triple vaccine (diphtheria tetanus and pertussis absorbed) was obtained from the Lister Institute Elstree Hearts England Each dose of this preparation contained 30 Lf purified diphtheria toxoid 6 Lf purified tetanus toxoid and 20 000 million *Bordetella pertussis* in 0 5 ml

Tetanus and diphtheria antitoxin titration

The tetanus antitoxin titer was determined by an indirect haemagglutination test as described by Fulthorpe (3) Sheep red blood cells treated with tannic acid were sensitized with tetanus toxoid obtained from the Austrian Institute for Haemoderivate Vienna Antidiphtheria antitoxin levels were also titrated by indirect haemagglutination (17) Sheep red blood cells treated with tannic acid were sensitized with diphtheria toxoid obtained from the Austrian Institute for Haemoderivate Vienna For both titrations the first serum dilution was 1 10

Immunoglobulins quantitation

Immunoglobulin levels were determined by the single radial immunodiffusion test (5) using antibody containing plates purchased from Meloy Laboratories (Springfield Virginia) and locally prepared plates Commercial standards from Meloy Laboratories were used along with international standards obtained from the WHO International Reference Centre for Immunoglobulins Lausanne Switzerland (10)

RESULTS

The mean values of the antitetanus and antidiphtheria for the control and malnourished children are given in Table 1 The data were converted from a nonlinear to a linear scale by expressing the results as log² of titres for statistical analysis Using the *t* test there was no significant difference ($p>0$ 05) between the marasmic the moderateley malnourished and the control group in their primary or secondary responses to either tetanus or diphtheria The tertiary response to diphtheria immunization of both marasmic and moderately malnourished children was significantly higher ($p<0$ 05) than that for the control group The tertiary response of marasmic children to tetanus was significantly higher than that of the control group ($p<0$ 05) while that of the mod

Table 2 *Mean immunoglobulin levels in malnourished children before and after treatment as compared to controls*

		No of children	% weight for age	Level (g/litre) IgG	IgA	IgM
Marasmus	Before recovery	10	55 0± 4 4	5 75±2 47	0 34±0 23	1 61±0 42
	After recovery	10	77 1± 4 4	7 33±3 43	0 28±0 15	1 52±0 82
Moderate PCM	Before recovery	11	75 1± 5 9	6 52±2 52	0 29±0 19	1 17±0 55
	After recovery	11	88 1±10 9	8 67±3 44	0 24±0 10	1 07±0 35
Control		35	103 0± 7 0	5 69±2 80	0 25±0 19	0 98±0 50

erately malnourished group was not significantly different ($p>0\ 05$)

The mean levels of IgG IgA and IgM immunoglobulins are given in Table 2 Since immunoglobulins levels in infants are age dependent the statistical analysis was done by the analysis of covariance (16) There were no significant differences ($p>0\ 05$) between the control the marasmic and the moderately malnourished groups for the three immunoglobulin classes IgG IgA and IgM at the start of the treatment or after recovery Furthermore there was no significant difference ($p>0\ 25$) between any of the three immunoglobulin levels at the start of treatment and after recovery

DISCUSSION

It has been suggested that the most important two factors that determine the well being of infants in any community are nutritional status and freedom from disease A combination of malnutrition and infections is responsible for the high infant mortality rate in developing countries (8 13) In communities where malnutrition is common there is no clear recommendation as to whether children suffering from malnutrition should be vaccinated In this country and perhaps in other developing countries there is an impression that vaccinating a malnourished child may not be advisable and that it may even be harmful since the response of such children may not be adequate In this respect there is nothing in our data to suggest that marasmic children should not be vaccinated with the triple vaccine since such children are capable of responding normally to this vaccine provided that they are being treated for malnutrition

ACKNOWLEDGEMENT

This work was supported by a grant from WHO and in part by a grant from the Lebanese National Council for Scientific Research

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Submitted Jan 19 1977
Accepted March 25 1977

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Acta Pædiatr Scand 66 693–698 1977

HEMOLYTIC UREMIC SYNDROME

Results of Treatment with Hemodialysis

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ABSTRACT Ekberg M Holmberg L and Denneberg T (Department of Paediatrics and the Renal Unit Department of Internal Medicine I University of Lund General Hospital Malmo Sweden) Hemolytic uremic syndrome Results of treatment with hemodialysis Acta Paediatr Scand 66 693 1977.—The characteristics of the hemolytic uremic syndrome in 7 children living in a well defined area in the south of Sweden are described All the patients had a severe form of the disease and were critically ill The clinical activity could best be followed by measuring blood platelets and urinary FDP Early institution of hemodialysis treatment given almost daily until normalisation of platelet count and urinary output is the most important live-saving measure Full dosage heparin seems not to be necessary Six patients survived and were followed up for 1–7 years When last seen they all had normal renal function and blood pressure

KEY WORDS Hemolysis thrombocytopenia renal insufficiency urinary FDP hemodialysis heparin

The hemolytic uremic syndrome (HUS) is characterised by acute renal failure hemolytic anemia and thrombocytopenia In many parts of the world HUS is now recognised as the most common cause of acute renal insufficiency in children (9 22) In some regions the disease tends to be rather mild with a low mortality (11 19 28) while in others the mortality is considerable (13 20 29) The etiology of the syndrome is unknown and its treatment largely empirical and controversial This paper reports the results of a treatment without full dosage heparin and steroids but with prompt hemodialysis in the event of progressive renal insufficiency

CLINICAL MATERIAL

The material consisted of 7 children (5 girls and 2 boys) treated in the Department of Paediatrics at Malmö General Hospital 1970 1976 Four of the children were from the city of Malmö (250 000 inhabitants) while the remaining 3 were referred to us from other hospitals in the area (with about 1 1/2 million inhabitants) The youngest patient was 12 months old at clinical onset and the next youngest was 14 months Two patients were 2 years 4 months and 7 years 6 months respectively Three patients were around 7 years The mean age was 4 years 10 months The clinical and laboratory findings in the patients are presented in Table 1

METHODS

Dialysis treatment Disposable dialysers with a total surface of 0 16–0 3 m² were used for hemodialysis The connecting tubes and the dialyser which had a total volume of less than 170 ml were primed with fresh blood because of the anemia and thrombocytopenia Each unit of blood (450 ml) contained 500–1 000 I U heparin The duration of each hemodialysis was 2–4 hours As a rule treatment was given every day during the period of oliguria anuria The degree of ultrafiltration was varied according to the desired weight loss during the hemodialysis treatment

Cannulation The silastic teflon arteriovenous cannula system described by Quinton et al (25) was placed in the radial artery and in the cephalic vein In one patient the brachial artery was cannulated instead

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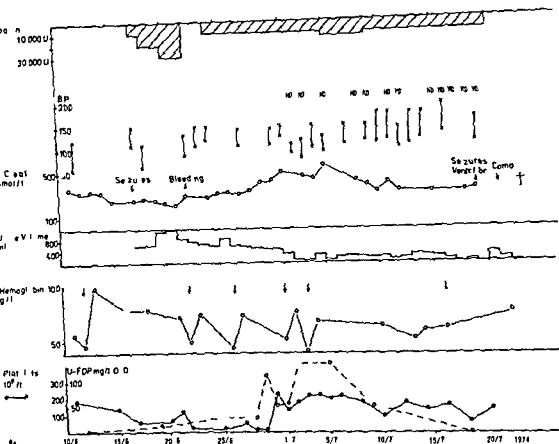


Fig 1 A 7 year old girl with HUS She was first treated with hydrocortisone and prednisolone (100 mg daily) be tween the 13th and 17th of June *On the 17th of June she* got seizures the blood pressure was *150/100 and the* corticosteroids were withdrawn She then *received full* dosage heparin for 2 weeks During this period serum *creatinine rose* urinary output fell successively and severe hypertension appeared Hemodialysis was started on the 3rd week Despite daily HD and intensive anti hypertensive therapy the blood pressure could not be *controlled* and she died from intracranial hemorrhage

sure was raised Treatment with full dosage heparin was started but the urine output nevertheless fell progressively and the serum creatinine gradually increased The platelet count decreased and urinary fibrinogen/fibrin degradation products (FDP) appeared Hemodialysis was started on the 22nd day after onset Intensive therapy because of severe hypertension was given with various combina tions of hydralazine chlorpromazine clonidine reserpine propranolol and trimetha phan (Arfonad) Treatment was not success ful and the child died from an intracranial hemorrhage Autopsy showed hyperplastic in timal thickening with markedly narrowed lumina of renal afferent arterioles and small ar teries Scattered subendothelial deposits of fibrinoid were present but nowhere were fibrin or platelet thrombi found There was widespread glomerular damage with patchy necrosis endothelial cell proliferation and in crease of mesangial matrix The tubuli were dilated and contained proteinaceous and blood cell casts The histological changes were con fined to the kidneys There was a hematoma in the chorioid plexus of the 4th ventricle and diffuse subarachnoidal hemorrhage but no vascular abnormalities in the CNS were found

Some complications in the 7 patients could be ascribed at least partly to the treatment

Table 1 *Laboratory findings physical signs and symtoms on the day of admission in the seven patients with HUS*

	Case 1 ♂ 7 y	2 ♀ 7 y	3 ♀ 2½ y	4 ♀ 2 y	5 ♂ 2 y	6 ♀ 1 y	7 ♀ 1 y	Normal range
Laboratory results								
Hb (g/l)	41	82	69	59	48	73	78	102–140
Platelets (10^9/l)	30	50	20	40	80	14	26	175–340
Haptoglobin (g/l)	0 03	0	0	0	0	0	0	0 3–2 0
S creatinine (μmol/l)	884	256	354	884	1 000	212	362	60–115
Urine volume (l/24 h)	0	0 6	0 2	0 03	0	0 03	0	
Physical signs and symptoms								
Pallor	+	+	+	+	+	+	+	
Petechiae/ecchym	−	−	−	−	−	−	+	
Diarrhea	+	+	+	+	+	−	+	
Bloody stools	+	−	+	−	−	−	−	
Seizures	−	−	−	+	−	−	+	
Edema	−	−	+	+	+	−	−	
Hypertension	−	+	−	−	−	+	+	

Parenteral nutrition During the acute stage all patients had an indwelling intravenous catheter by which sugar solutions (10% Invertos) and essential amino acids (Kidnamin Vamin) could be given when necessary Many of the patients could be fed by mouth after vomiting had subsided

Heparin treatment Two patients got no heparin except the small amounts received during hemodialysis Four patients received small doses intermittently or continuously between the dialyses (50–100 I U /kg/24 h) mainly to prevent clotting in the Scribner shunt The dosage was such that the whole blood clotting time and the activated partial thromboplastin time were generally not affected Only one patient was fully heparinised (APTT 80–120 sec)

Blood transfusions Fresh blood was given in association with the dialysis treatment and packed red cells between the treatments when the hemoglobin fell below 60 g/l

RESULTS

The details of the treatment are summarised in Table 2 Six patients were treated with hemodialysis within 5 days of the appearance of signs of hemolytic anemia thrombocytopenia or renal failure The patients received dialysis on 5–8 occasions The maximal duration of anuria was 10 days Two of these patients got no heparin at all between the dialyses and 4 received only small amounts not enough to affect the clotting time or the APT time All 6 patients survived Four of them initially had hypertension which was controlled either by dialysis alone or combined with dihydralazine and reserpine

One of the 7 patients died (Table 2) from intracranial hemorrhage The clinical course is illustrated in Fig 1 A 7 year old girl fell ill with vomiting and diarrhea She soon developed profound anemia hepatomegaly and jaundice Serum creatinine was increased but she had no oliguria in the beginning One week after onset she was referred to our hospital because of convulsions The blood pres

Table 2 *Treatment*

No of patients	Time before first dialysis	No of dialyses	Heparin treatment	No of blood transfusions	Duration of anuria/oliguria
6	<5 days	5–8	None or low dose	3–9	5–10 days
1 †	22 days	12	500–1 500 IU/ kg/24 h	3	27 days

severely damaged Urinary FDP which form on lysis of fibrin deposits in the kidneys appeared late in this patient which might indicate that she had had a weak fibrinolytic defence Although neither fibrin nor platelet thrombi could be found at autopsy 3 weeks later she might not have been able to break down the arteriolar and glomerular thrombi quickly enough In patients like this i e in whom the fibrinolytic capacity is not sufficient to cope with the stress imposed upon it the prognosis may be gloomy However we have so far refrained from thrombolytic treatment because of the serious bleeding complications reported (23)

The etiology of HUS is unknown The search for environmental factors has generally been unrewarding One of our cases showed a significant rise in titre of complement fixing antibodies against Mycoplasma pneumoniae but in none of the others could we detect any clue to the etiology The complement factors were determined in 3 patients C3 was decreased in 2 C4 in 1 and C1q in 1 This suggests that in some cases the initial vessel damage might be mediated through complement activation by an antigen antibody reaction But judging also from some recent reports (5 15 21 27) complement activation occurs in only some of the cases Immunofluorescence studies of renal biopsy specimens have often failed to demonstrate immunoglobulins or complement in the glomerular capillaries (8 12 26) The evidence for immunological mechanisms in HUS is thus weak

The final outcome in HUS seems to vary geographically In Argentina HUS has become an important cause of chronic renal disease in children (9) This is not the case in some other regions (28) including Scandinavia One of our patients who had severe hypertension died while all the others recovered with seemingly normal renal function The prognosis thus seems to depend more upon the severity of the hypertension than upon other factors such as the degree of hemolysis or the duration of oliguria anuria

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urosepsis after catheterisation of the bladder in 1 case wound sepsis from the site of the Scribner shunt in 1 case and bleeding during heparin treatment in 2 Two patients had sequelae after the cannulation One patient got paresis of the median and ulnar nerves The paresis was mild and at follow up there was practically no disability The second patient developed gangrene of the fourth finger which had to be amputated

When last seen 1–7 years after recovery all 6 surviving patients had normal renal function and blood pressure

DISCUSSION

Early hemodialysis is in our opinion essential for successful management of the hemolytic-uremic syndrome (7) Many patients present with overhydration and hypertension leading to seizures The intensive hemolysis accentuates the tendency to hyperkalemia which is easily controlled by hemodialysis All the 6 patients who were dialysed within 5 days recovered They were all critically ill on admission The vital importance of early dialysis has repeatedly been stressed (13 15) Peritoneal dialysis has often been used but we have found that hemodialysis is advantageous although it sometimes involves technical problems in small children New types of shunts and filters especially designed for small children have reduced these technical difficulties (16 17) We perform hemodialysis practically every day during the period of oliguria anuria Abundant amino acids and carbohydrate can then be given to the patients without any risk of overloading the circulation In this way the catabolic phase can be shortened Furthermore hemodialysis which takes only 2–4 hours at a time permits early mobilisation in the intervals between the dialyses as well as earlier oral feeding We feel that it is essential to keep the patient in a good general condition throughout the treatment to avoid complications such as bacterial sepsis

Hemolytic anemia is always present in the acute stage of HUS The invariable finding of a negative Coombs test and the typical morphologic appearance of the red cells clearly suggest a non immune acquired hemolytic anemia (3) In such a type of anemia the red cells are thought to be injured during their passage through regions of damaged and obstructed small vessels (4) and not because of an immunological mechanism Treatment of the anemia with corticosteroids is thus theoretically not justified Only one of our patients the one who died received a short course of treatment with prednisone Neither have other workers found any practical value of corticosteroids which thus should be avoided (18)

Biopsy and autopsy studies make it likely that the primary lesion in HUS is a swelling of the endothelial cells of renal arterioles and glomerular capillaries (8 12 20 31) with consequent narrowing of the lumen A secondary accumulation of platelets and fibrin forming either occlusive thrombi (6, 9 20) or subendothelial deposits (8 26 31) seems to play an important role in the pathogenetic process The use of heparin to prevent thrombus formation has been advocated but is controversial (10 13 24 28 30) In our study only one patient was fully heparinised but she died It is difficult to imagine that the small doses received by some of the other patients would have affected the outcome Thus in our experience heparin is not indicated in HUS It might even do harm because of bleeding

It is possible that thrombolytic agents (2 23) or platelet aggregation inhibitors (1) would have been better than heparin in the case with a fatal outcome From Fig 1 it is evident that the decrease in the patient s urinary output was preceded by a few days drop in the platelet count The platelet count remained low during the whole phase of deterioration after which it suddenly returned to normal without any other improvement in her condition This might mean that the pathogenetic mechanism of platelet and fibrin deposition ceased at this point but the kidneys had already been too

severely damaged Urinary FDP which form on lysis of fibrin deposits in the kidneys appeared late in this patient which might indicate that she had had a weak fibrinolytic defence Although neither fibrin nor platelet thrombi could be found at autopsy 3 weeks later she might not have been able to break down the arteriolar and glomerular thrombi quickly enough In patients like this i e in whom the fibrinolytic capacity is not sufficient to cope with the stress imposed upon it the prognosis may be gloomy However we have so far refrained from thrombolytic treatment because of the serious bleeding complications reported (23)

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Submitted Dec 22 1976
Accepted April 10 1977

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Acta Pædiatr Scand 66 699–703 1977

LACTOSE AND PROTEIN ABSORPTION FROM BREAST MILK AND COW'S MILK PREPARATIONS AND ITS INFLUENCE ON THE INTESTINAL FLORA

Investigations on two Infants with an Artificial Anus

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ABSTRACT Heine W Zunft H J Muller Beuthow W and Grutte F K (Department of Metabolic Diseases and Nephrology of the Paediatric Clinic of the Wilhelm Pieck University Rostock and Department of Microecology of the Central Institute of Nutrition Potsdam Rehbrucke GDR) Lactose and protein absorption from breast milk and cow s milk preparations and its influence on the intestinal flora Acta Paediatr Scand 66 699 1977 — Lactose and protein absorption from breast milk and a cow s milk preparation enriched up to 7% of lactose were studied in two infants with an artificial anus applied in the ascending colon region The concentrations of protein lactose glucose and galactose were measured in the fistula stools In addition the stools were analysed microbiologically There were relatively high concentrations of lactose and its decomposition products and low concentrations of protein and aminonitrogen in the fistula stools when breast milk was fed When the cow s milk formula was applied only traces of lactose but high amounts of protein were measured The microbiological findings are in agreement with the hypothesis that the bacterial flora of the large intestine is influenced by the lactose and protein concentrations in the intestinal content which reach the large intestine

KEY WORDS Mother s milk lactose absorption protein absorption intestinal flora

It is well known that feeding infants exclusively on breast milk has a specific effect on the appearance of the stools as well as on the composition of their microflora when compared with artificial feeding The faeces are characterized by higher bifidobacterial counts usually including a special type and low counts of putrefactive bacteria The reasons for this difference have been intensively studied (4–9 12 13 16 17 18 19) One of the motives for these studies was to create an artificial milk preparation producing the specific effects of breast milk This would be of practical value in view of the better resistance of breast fed babies against infection especially diarrhoea and in view of other advantages of natural infant nutrition and would reduce the severity of iron depletion among infants fed on artificial milk

In 1950 Gyorgy (8) found a type of bifidobacteria which was dependent on breast milk for its growth in vitro Many natural constituents of breast milk especially aminosaccharides have been tested for their ability to produce a bifidogenic intestinal flora but without success (7) An important step was the discovery by Petuely (16) that lactulose enhances the bifidus flora when added to cow milk preparations

Breast milk however does not contain lactulose (8) and the types of bifid organisms in stools of breast fed infants may differ from those in stools of infants fed with lactulose

The specific effects of the lactose and pro-

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Submitted Dec 22 1976
Accepted April 10 1977

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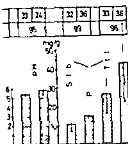


Fig. pH, soluble protein, total protein. n = number of faeces samples, % = significance level of differences (Student's t-test)

RESULTS

The biochemical parameters of the stool specimens differed markedly between the two feeding systems. Although the KtNa formula was enriched to contain 7% lactose, this sugar was completely absorbed, whereas an equicaloric breast milk diet resulted in stools containing relatively high amounts of lactose (Fig. 1). Glucose and galactose concentrations differed in a similar manner: when breast milk was given, the stools contained higher galactose and glucose concentrations than when obtained during formula feeding. In contrast to these findings, the stools obtained on a breast milk diet had considerably lower α amino nitrogen and protein concentrations (Figs. 1 and 2). The pH values were low during both feeding systems, but they were slightly lower for breast milk than for the cow's milk preparation.

Higher total counts of viable bacteria were found in the fistula stools obtained by formula feeding (Fig. 3). The numbers of all types of bacteria increased: *Escherichia coli*, *Klebsiella*, *Proteus*, *Lactobacillus* and *Enterococcus*, *Bifidobacterium* and *Bacteroides*.

DISCUSSION

This investigation was performed in order to prove the hypothesis that the microbiological and physicochemical properties of faeces obtained on a breast milk diet depend on delayed lactose absorption and on almost complete absorption of protein in the small intestine.

The lactose and protein concentrations in ordinary stools resulting from breast milk and cow's milk diets are very low, since the prolonged passage through and the intensity of the bacterial metabolism in the large bowel will result in more or less complete utilization of these nutrients (3). Hence, to measure the absorption in the small intestinal tract it is necessary to determine the concentrations of these substances before bacterial decomposition takes place, i.e. when the intestinal content passes the ileocaecal valve.

Our results from studying two infants with an artificial anus in the region of the ascending colon support the hypothesis of lactose from breast milk being less well absorbed than that in our cow's milk preparation. Though equal amounts of lactose were given during both feeding regimens, the lactose concentration in the fistula stools was 169 times higher during breast milk feeding than when the cow's milk preparation was applied. Similar results were obtained by Hirata et al. (12) using an intestinal intubation technique which allowed the analysis of samples of the intestinal content obtained from just above the ileocaecal region. They found higher lactose concentrations during breast milk feeding than during feeding with cow's milk preparations, even when these were enriched to contain 15% lactose. Our experiments revealed similar results for glucose and galactose, the prod

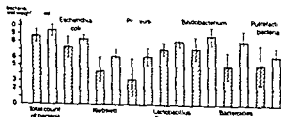


Fig. 3. Microbiological findings in stools from two infants with an artificial anus. The infants were fed either on breast milk (shaded column) or a lactose enriched formula (dotted column). Mean values from 18 faeces samples of the breast milk group and 10 samples of the formula group. Significance level of differences between the two groups in all bacteria with exception of putrefactive bacteria: 99%

Table 1 *Composition of KiNa formula*

Protein	1 8 g/100 ml
Fat	3 3 g/100 ml
Total carbohydrates	7 8 g/100 ml
Lactose	4 0 g/100 ml
Minerals	0 4 g/100 ml
Sodium	28 mg/100 ml
Potassium	70 mg/100 ml
Calcium	60 mg/100 ml
Iron	1 2 mg/100 ml
Copper	0 07 mg/100 ml
Caloric value	70 calorie /100 ml
Vitamin A	190 IU/100 ml
Vitamin B_1	0 06 mg/100 ml
Vitamin B_2	0 1 mg/100 ml
Vitamin B_6	0 06 mg/100 ml
Vitamin C	45 mg/100 ml

tein content the phosphate concentration buffer capacity and other qualities of milk on the intestinal flora are still under discussion (4 6 22) Bullen and coworkers have suggested that the intestinal flora is related to the bio chemical milieu (4*a* 4*b* 22) Grutte Haenel and coworkers suggested that the passage of lactose through the intestinal tract may be con nected with the antiputrifactive action of breast milk (5 6 20) According to their hypo thesis the lactose in cow s milk and its pre parations is completely absorbed This may be due to certain factors for instance phosphate ions promoting the process of mutarotation of β lactose to α lactose the latter being more easily absorbed In breast milk mutarotation is not influenced in this way because of lower concentrations of promoting factors Hence a certain quantity of lactose remains unabsor bed reaches the large intestine and can here be metabolized by the microflora This causes an acid milieu which inhibits putrefactive processes

In view of this hypothesis it would be of interest to compare the absorption of lactose protein and α aminonitrogen from breast milk and from cow s milk preparation in the course of the chyme transport through the small in testinal bowel For this reason lactose and nitrogen balance studies were performed in different feeding regimens on two infants each with an artificial anus provided in the region of the ascending colon as a consequence of in testinal malformations The results obtained were related to the microbiological findings in the stools from the artificial anus (fistula stools)

METHODS

Both infants studied were 4 months old Pregnancy and delivery were uncomplicated Their birth weights were 3280 g and 3060 g respectively Immediately after birth an atresia of the anus was noted in both infants Laparotomy was performed and an artificial anus was applied on the first day of life The further progress of the infants was uneventful Initial feeding on breast milk and later on KiNa formula feeding resulted in an adequate gain in body weight When lactose absorption studies were per formed the general condition of both infants was good and their body weights were about 4 500 g and 4 800 g respectively

For 10 days they were given breast milk at rates of 5×170 ml and 5×160 ml respectively The fistula stools at that time had the typical appearance of stools from breast fed babies The fistula stools were collected every 4 hours over a period of 48 hours The lactose glucose galactose protein and α aminonitrogen concentrations in these stools were determined and microbiological ana lyses were undertaken

The nutrition was then changed to an equicaloric cow s milk formula diet (KiNa) containing 1 8% protein and enriched with lactose up to 7% The actual composition of this formula is given in Table 1 Fourteen days later stool were again examined as described above

Glucose and galactose in stool specimes were deter mined enzymologically according to the method of Berg meyer & Bernt (2) The lactose concentration was cal culated by incubation of stool specimens in phosphate buffer with β galactosidase at pH 7 0 and then measuring the increase in the glucose concentration Protein was controlled according to Lowry (15) α amino-N according to Antener (1) Microbiological investigations were per formed after the methods of Haenel et al (10)

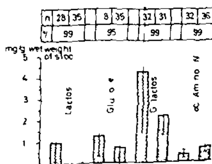


Fig 1 Biochemical findings in stools from two infants with an artificial anus The infants were fed either on breast milk (shaded column) or a lactose-enriched formula (dotted column) (Lactose glucose galactose α amino-N concentration) n=number of faeces samples % = sig nificance level of differences (Student s t test)

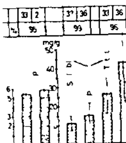


Fig. 2. pH, soluble protein, total protein. n=number of faeces samples. %=significance level of differences (Student t test).

RESULTS

The biochemical parameters of the stool specimens differed markedly between the two feeding systems. Although the KiNa formula was enriched to contain 7% lactose, this sugar was completely absorbed, whereas an equicaloric breast milk diet resulted in stools containing relatively high amounts of lactose (Fig. 1). Glucose and galactose concentrations differed in a similar manner: when breast milk was given, the stools contained higher galactose and glucose concentrations than when obtained during formula feeding. In contrast to these findings, the stools obtained on a breast milk diet had considerably lower α amino nitrogen and protein concentrations (Figs. 1 and 2). The pH values were low during both feeding systems, but they were slightly lower for breast milk than for the cow's milk preparation.

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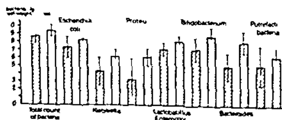


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Table 1. *Composition of KiNa formula*

Protein	1.8 g/100 ml
Fat	3.3 g/100 ml
Total carbohydrates	7.8 g/100 ml
Lactose	4.0 g/100 ml
Minerals	0.4 g/100 ml
Sodium	28 mg/100 ml
Potassium	70 mg/100 ml
Calcium	60 mg/100 ml
Iron	1.2 mg/100 ml
Copper	0.07 mg/100 ml
Caloric value	70 calories/100 ml
Vitamin A	190 IU/100 ml
Vitamin B_1	0.06 mg/100 ml
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In view of this hypothesis, it would be of interest to compare the absorption of lactose, protein and α-aminonitrogen from breast milk and from cow's milk preparation in the course of the chyme transport through the small intestinal bowel. For this reason, lactose and nitrogen balance studies were performed in different feeding regimens on two infants, each with an artificial anus provided in the region of the ascending colon as a consequence of intestinal malformations. The results obtained were related to the microbiological findings in the stools from the artificial anus (fistula stools).

METHODS

Both infants studied were 4 months old. Pregnancy and delivery were uncomplicated. Their birth weights were 3 280 g and 3 060 g respectively. Immediately after birth an atresia of the anus was noted in both infants. Laparatomy was performed and an artificial anus was applied on the first day of life. The further progress of the infants was uneventful. Initial feeding on breast milk and later on KiNa formula feeding resulted in an adequate gain in body weight. When lactose absorption studies were performed, the general condition of both infants was good and their body weights were about 4 500 g and 4 800 g respectively.

For 10 days they were given breast milk at rates of 5×170 ml and 5×160 ml respectively. The fistula stools at that time had the typical appearance of stools from breast fed babies. The fistula stools were collected every 4 hours over a period of 48 hours. The lactose, glucose, galactose, protein and α-aminonitrogen concentrations in these stools were determined and microbiological analyses were undertaken.

The nutrition was then changed to an equicaloric cow's milk formula diet (KiNa) containing 1.8% protein and enriched with lactose up to 7%. The actual composition of this formula is given in Table 1. Fourteen days later stools were again examined as described above.

Glucose and galactose in stool specimens were determined enzymologically according to the method of Bergmeyer & Bernt (2). The lactose concentration was calculated by incubation of stool specimens in phosphate buffer with β-galactosidase at pH 7.0 and then measuring the increase in the glucose concentration. Protein was controlled according to Lowry (15), α-amino N according to Antener (1). Microbiological investigations were performed after the methods of Haenel et al. (10).

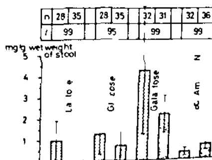


Fig. 1. Biochemical findings in stools from two infants with an artifical anus. The infants were fed either on breast milk (shaded column) or a lactose enriched formula (dotted column). (Lactose, glucose, galactose, α-amino-N concentration.) n = number of faeces samples. % = significance level of differences (Student's t test).

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Submitted Sept 8 1976
Accepted April 13 1977

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ucts of lactose decomposition there were considerably higher concentrations in the fistula stools obtained from breast milk than in those obtained after feeding on cow s milk this showing that lactose is also split by bacterial activity

In contrast the concentrations of α amino nitrogen and of soluble and total protein in the fistula stools were nearly twice as high during the cow s milk period than during the breast milk period although the amount of protein given daily in the cow s milk was only 1 25 times larger than that contained in the breast milk diet Similar differences in nitrogen concentrations have already been shown between ordinary stools obtained by breast milk feeding and feeding on cow s milk preparations (11)

The reverse situation of protein and lactose digestion/absorption cannot be explained simply by the more rapid passage through the intestinal tract when breast milk is used for feeding Even if the lactose concentration in cow s milk preparations is increased to 7% and more no anti putrefactive bacterial flora will develop It seems more likely that the degree of mutarotation of lactose and its effect on the digestibility of this sugar is the main factor influencing the absorption of lactose

The differences in the biochemical composition of the intestinal contents reaching the colon is probably the cause of the differences in the microbiological findings The total bacterial counts and the counts for all of the different bacterial groups in the fistula stools were higher during application of the cow s milk preparation than during breast milk feeding The composition of the intestinal flora in infants fed on breast milk as determined in stool cultures and characterized by lower counts for putrefactive bacteria and higher counts for bifidobacteria were not confirmed by the microbiological analysis of the fistula stools This indicates that the typical changes appear during the passage of the intestinal contents through the large bowel A tendency to low putrefactive bacteria counts was however recognizable in the fistula stools (Fig 2) this indicating the major importance of a high lactose—low protein concentration for the suppression of putrefactive germs The preponderance of bifidobacteria on the other hand would appear to be a secondary phenomenon

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Submitted Sept 8 1976
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Acta Pædiatr Scand 66 705–708 1977

ANTIBODY PRODUCTION BY THE MAMMARY GLAND IN MOTHERS AFTER ARTIFICIAL ORAL COLONISATION OF THEIR INFANTS WITH A NON PATHOGENIC STRAIN *E COLI* 083

R LODINOVÁ and V JOUJA

From the Research Institute for the Care of Mother and Child Prague Czechoslovakia

ABSTRACT *Lodinova R* and Jouja V (Research Institute for the Care of Mother and Child Prague Czechoslovakia) Antibody production by the mammary gland in mothers after artificial oral colonisation of their infants with a non pathogenic strain *E coli* 083 Acta Paediatr Scand 66 705 1977.—Twenty five breast fed and 25 formula fed infants were colonised by oral administration of a living suspension of *E coli* 083 Twenty breast fed and 13 formula fed infants were followed as controls Specific antibody titres in serum stool filtrates and milk and secretory IgA levels in stool filtrates and milk were determined in samples taken fortnightly from birth until 20 weeks of age The haemagglutinating antibody in serum and milk increased in the colonised groups but in stool filtrates an inhibitory effect of breast milk was demonstrated Secretory IgA levels in stool filtrates were significantly higher in colonised infants and breast fed controls than in bottle fed infants during the period of breast feeding Then levels in the colonised groups remained high but in breast fed controls they decreased to values found in bottle fed controls Artificial colonisation evoked local antibody and secretory IgA responses in the intestine as well as an antibody response in the mother s mammary gland The possible protective effect of those responses is discussed

KEY WORDS *Artificial E coli* colonisation antibody response mammary gland intestine sIgA

Over the past 20 years neonatal morbidity and mortality have rapidly decreased in industrialised countries in spite of the fact that the incidence of breast feeding has markedly decreased This might suggest that artificial food can fully substitute for maternal milk

Recently several reports have shown a changing pattern with an increased incidence of infection caused by gram negative bacteria (6)

Since breast milk antibodies are not absorbed from the intestine their protective role has been doubted (13) According to recent views secretory IgA has been considered to be the dominant immunoglobulin of human milk and other secretions as well as the mediator of local immunity (5 8) Secretory IgA may block adherence of bacteria to the intestinal mucosa and influence intestinal flora by selecting mutants of lower virulence (7) The survival of maternal antibodies in the neonatal gut has been attributed to the intrinsic resistance of secretory IgA to trypsin digestion (11)

The problem of increasing gastrointestinal infection due in many cases to *E coli* strains resistant to most antibiotics has become a general one This has stimulated our attempts to find a method for protecting bottle fed infants in a physiological manner

In the present study the influence of artificial colonisation with the non enteropathogenic *E coli* strain 083 used in our previous

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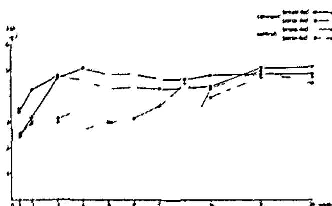


Fig. 1. Specific haemagglutinating antibody against *E. coli* 083 in serum. Group 1: Breast fed colonised infants. Group 2: Bottle fed colonised infants. Group 3: Breast fed controls. Group 4: Bottle fed controls. Level of significance: 6 weeks: 1: 3+4 $p<0.05$, 2: 3+4 $p<0.05$; 8 weeks: 1: 3+4 $p<0.05$, 2: 3+4 $p<0.05$; 10 weeks: 1: 3+4 $p<0.05$, 2: 3+4 $p<0.05$; 12 weeks: 1: 3+4 $p<0.05$, 2: 3+4 $p<0.05$.

work (9) on local antibody and secretory IgA responses in the intestine and mammary gland was investigated.

MATERIALS AND METHODS

Twenty five breast fed and 25 formula fed infants were colonised by oral administration of the non pathogenic *E. coli* strain 083. A living suspension was prepared from a 24 hr culture containing 5×10^{8} organisms/ml. One ml was given to each child during the first 24 hr after birth and again 3 times a week for four successive weeks. Twenty breast fed and 13 formula fed infants were fol-

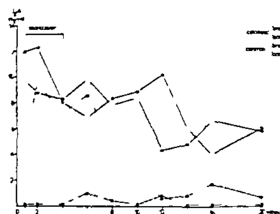


Fig. 3. Secretory IgA levels in stool filtrates. Group 1: Breast fed colonised infants. Group 2: Bottle fed colonised infants. Group 3: Breast fed controls. Group 4: Bottle fed controls. Significancy: 2 weeks: 1: 4 $p<0.01$, 2: 4 $p<0.01$, 3: 4 $p<0.01$; 4 weeks: 1: 4 $p<0.05$, 2: 4 $p<0.05$, 3: 4 $p<0.05$; 6 weeks: 1: 4 $p<0.05$, 2: 4 $p<0.05$, 3: 4 $p<0.05$; 8 weeks: 1: 3 $p<0.1$, 1: 4 $p<0.05$, 2: 4 $p<0.05$; 10 weeks: 1: 3 $p<0.05$, 1: 4 $p<0.5$, 2: 3 $p<0.05$, 2: 4 $p<0.05$, 3: 4 $p<0.05$; 12 weeks: 1: 3 $p<0.05$, 1: 4 $p<0.1$, 2: 3 $p<0.05$, 2: 4 $p<0.05$.

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Blood and stool samples were taken every fortnight until the 16th week and then in the 20th and 24th weeks of life. In 4 mothers from the control group and 17 mothers from the colonised group milk samples were taken every fortnight during breast feeding. Separate milk samples taken from 10 mothers in the second week after delivery were added to the control group. Stool samples from all mothers and infants were taken before colonisation and every fortnight up to 4–6 months. Smears from the infant's

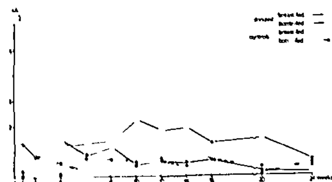


Fig. 2. Specific haemagglutinating antibody against *E. coli* 083 in stool filtrates. Group 1: Breast fed colonised infants. Group 2: Bottle fed colonised infants. Group 3: Breast fed controls. Group 4: Bottle-fed controls. Level of significance: 2 weeks: 1: 3 $p<0.1$, 1: 4 $p<0.05$, 2: 4 $p<0.1$; 4 weeks: 1: 3 $p<0.1$, 1: 4 $p<0.05$, 2: 3 $p<0.05$, 2: 4 $p<0.05$; 10 weeks: 2: 1 $p<0.01$, 2: 3 $p<0.05$, 2: 4 $p<0.05$; 14 weeks: 2: 1 $p<0.05$, 2: 3 $p<0.05$, 2: 4 $p<0.05$; 20 weeks: 2: 1 $p<0.05$, 2: 3 $p<0.05$, 2: 4 $p<0.05$.

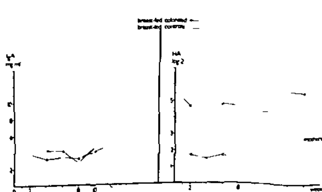


Fig. 4. Secretory IgA levels in breast milk. Haemagglutinating antibody against *E. coli* 083 in breast milk. Group 1: Breast milk from mothers of colonised infants. Group 2: Breast milk from mothers of controls. Significancy: Secretory IgA—not significant. Haemagglutinating antibodies: 2 weeks: 1: 2 $p<0.01$; 4 weeks: 1: 2 $p<0.01$; 8 weeks: 1: 2 $p<0.01$. Dashed line represents mean of 10 milk samples.

mouth were taken 4 hours after administration of the suspension of *E coli* once a week for four successive weeks i e during the time of colonisation

Specific antibodies against *E coli* 083 in serum stool filtrates and milk were determined by haemagglutination technique (10) secretory IgA levels in stool filtrates and milk by radial immunodiffusion (7) the presence of *E coli* 083 was detected bacteriologically as described in our previous work (9) Secretory IgA was not tested for specificity

For statistical evaluation the chi square test and the Fisher s test (3) were used

RESULTS

Titres of specific haemagglutinating antibodies against *E coli* 083 in the sera of colonised infants were significantly higher than in the control groups between the 6th to 12th weeks of life i e from the 2nd to 8th weeks after the end of colonisation After that period the titres of all groups did not differ (Fig 1)

In stool filtrates the titres of coproantibodies were higher in colonised bottle fed and breast fed infants between the 2nd to 4th weeks than in controls In colonised bottle fed infants the titres remained significantly higher in the 10th 14th and 20th weeks than in controls in colonised breast fed infants after 4 weeks the titres decreased (Fig 2)

Secretory IgA levels in stool were significantly higher in colonised bottle fed and breast fed infants and in breast fed controls than in the bottle fed control group in the 2nd 4th 6th 8th and 10th weeks Following this levels of secretory IgA in breast fed controls decreased and reached about the same low values found in artificially fed controls In both colonised groups however titres remained higher in the 14th and 20th weeks than in both control groups (Fig 3)

Antibody levels against *E coli* 083 in milk samples from mothers of colonised infants were significantly higher in the 2nd 4th and 8th weeks than in milk from mothers whose infants were not colonised (Fig 4)

Secretory IgA in maternal milk was not influenced by colonisation (Fig 4) *E coli* 083 was repeatedly found in the mouth of colonised infants and in their mothers milk but never in the mothers stools (will be published)

DISCUSSION

The serum antibody response after artificial colonisation was not influenced by breast feeding Rauss et al (12) also showed a systemic response after oral vaccination against dysentery even though the local coproantibody production was higher than in serum

Levels of secretory IgA in colonised formula fed and breast fed infants were high from the first week and remained high over the whole period of investigation However in the breast fed group passive transfer of secretory IgA via maternal milk cannot be distinguished from endogenous production In breast fed controls secretory IgA levels were also high during breast feeding and then decreased rapidly

The ability of the mammary gland to produce specific antibodies has also been shown by Allardyce et al (1) Antibodies increased in serum and colostrum after gastrointestinal infection caused by Salmonella during pregnancy Goldblum et al (4) used the same *E coli* strain as we for artificial colonisation of 3 pregnant women and showed by the plaque technique an appearance of colostral cells producing antibodies against the 0 antigen There was a lack of systemic immune reaction in these patients and a suggestion of a transfer of sensitised cells from the gastrointestinal tract into the mammary gland

In the present study only the infants were colonised Even in this arrangement the mammary gland has shown an antibody response but the mechanism is probably different It is suggested that the antibody producing cells of the mammary gland are stimulated by direct contact with antigen present in the infant s mouth

For protection against gastrointestinal infection artificial colonisation can substitute for the secretory IgA component of maternal milk and increase milk antibody levels

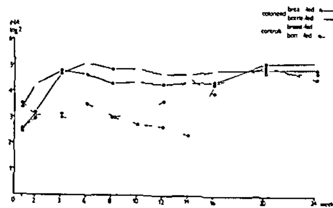


Fig 1 Specific haemagglutinating antibody against *E coli* 083 in serum Group 1 Breast fed colonised infants *Group 2 Bottle fed colonised infants Group 3 Breast fed* controls Group 4 Bottle fed controls Level of significance 6 weeks 1 3+4 $p<0.05$ 2 3+4 $p<0.05$ 8 weeks 1 3+4 $p<0.05$ 2 3+4 $p<0.05$ 10 weeks 1 3+4 $p<0.05$ 2 3+4 $p<0.05$ 12 weeks 1 3+4 $p<0.05$ 2 3+4 $p<0.05$

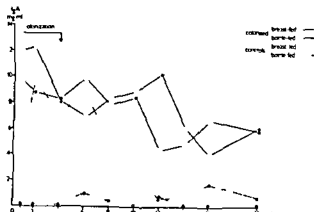


Fig 3 Secretory IgA levels in stool filtrates Group 1 Breast fed colonised infants Group 2 Bottle fed colonised infants Group 3 Breast fed controls Group 4 Bottle fed controls Significancy 2 weeks 1 4 $p<0.01$ 2 4 $p<0.01$ 3 4 $p<0.01$ 4 weeks 1 4 $p<0.05$ 2 4 $p<0.05$ 3 4 $p<0.05$ 6 weeks 1 4 $p<0.05$ 2 4 $p<0.05$ 3 4 $p<0.05$ 8 weeks 1 3 $p<0.1$ 1 4 $p<0.05$ 2 4 $p<0.05$ 10 weeks 1 3 $p<0.05$ 1 4 $p<0.5$ 2 3 $p<0.05$ 2 4 $p<0.05$ 3 4 $p<0.05$ 12 weeks 1 3 $p<0.05$ 1 4 $p<0.1$ 2 3 $p<0.05$ 2 4 $p<0.05$

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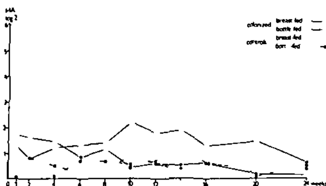


Fig 2 Specific haemagglutinating antibody against *E coli* 083 in stool filtrates Group 1 Breast fed colonised infants Group 2 Bottle fed colonised infants Group 3 Breast fed controls Group 4 Bottle fed controls Level of significance 2 weeks 1 3 $p<0.1$ 1 4 $p<0.05$ 2 4 $p<0.1$ 4 weeks 1 3 $p<0.1$ 1 4 $p<0.05$ 2 3 $p<0.05$ 2 4 $p<0.05$ 10 weeks 2 1 $p<0.01$ 2 3 $p<0.05$ 2 4 $p<0.05$ 14 weeks 2 1 $p<0.05$ 2 3 $p<0.05$ 2 4 $p<0.05$ 20 weeks 2 1 $p<0.05$ 2 3 $p<0.05$ 2 4 $p<0.05$

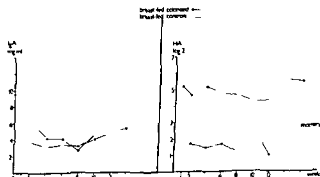


Fig 4 Secretory IgA levels in breast milk Haemagglutinating antibody against *E coli* 083 in breast milk Group 1 Breast milk from mothers of colonised infants Group 2 Breast milk from mothers of controls Significancy Secretory IgA—not significant Haemagglutinating antibodies 2 weeks 1 2 $p<0.01$ 4 weeks 1 2 $p<0.01$ 8 weeks 1 2 $p<0.01$ Dashed line represents mean of 10 milk samples

Acta Pædiatr Scand 66 709–712 1977

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From the Research Institute for the Care of Mother and Child Prague Czechoslovakia

ABSTRACT Lodinova R and Jouja V (Research Institute for the Care of Mother and Child Prague Czechoslovakia) Influence of oral lysozyme administration on serum immunoglobulin and intestinal secretory IgA levels in infants Acta Paediatr Scand 66 709 1977 — The influence of lysozyme feeding on the production of serum immunoglobulins and intestinal secretory IgA was studied in full term and premature infants from birth up to the age six months Serum immunoglobulins were not influenced by lysozyme administration An increase in secretory IgA was found in stool filtrates of full term lysozyme fed infants no secretory IgA was detected in controls In this way lysozyme feeding partly substituted for passive transfer of secretory IgA from maternal milk

KEY WORDS Lysozyme immunoglobulins secretory IgA

The difference in incidence of enteric infections between breast fed and bottle fed infants is well known A protective role of breast milk against enteric infections has been suggested from several observations (3 15) The establishment of secretory IgA as the predominant immunoglobulin in milk (10) offered new evidence on the protective role and importance of breast feeding

Another breast milk component—lysozyme—has also been detected in plasma saliva and tears (6 1) This enzyme is known to possess several biological activities closely connected with mechanisms protecting the organism against infection (5) Low levels found in small for date newborns may be partly responsible for their increased susceptibility to infection (11)

In breast milk the level of lysozyme is about mg/ml It modifies the digestive function of breast fed infants influences the character of their faeces and together with Bifidus factor promotes growth of *Lactobacillus bifidus* and inhibits that of *E coli* (5)

Thus bottle fed infants lack two important components of breast milk secretory IgA and lysozyme We have previously demonstrated that oral colonisation with a non enteropathogenic *E coli* strain evoked earlier and higher serum antibody and coproantibody responses as well as secretory IgA production in the intestine of artificially fed infants than in controls (13 14) In the present work we have studied the influence of lysozyme feeding on the production of serum immunoglobulins and secretory IgA in the intestine of artificially fed full term and premature infants as compared with control groups

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Accepted April 27 1977

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Submitted Sept 9 1976
Accepted April 27 1977

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Acta Pædiatr Scand 66 709–712 1977

INFLUENCE OF ORAL LYSOZYME ADMINISTRATION ON SERUM IMMUNOGLOBULIN AND INTESTINAL SECRETORY IgA LEVELS IN INFANTS

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From the Research Institute for the Care of Mother and Child Prague Czechoslovakia

ABSTRACT Lodinova R and Jouja V (Research Institute for the Care of Mother and Child Prague Czechoslovakia) Influence of oral lysozyme administration on serum immunoglobulin and intestinal secretory IgA levels in infants Acta Paediatr Scand 66 709 1977 — The influence of lysozyme feeding on the production of serum immunoglobulins and intestinal secretory IgA was studied in full term and premature infants from birth up to the age six months Serum immunoglobulins were not influenced by lysozyme administration An increase in secretory IgA was found in stool filtrates of full term lysozyme fed infants no secretory IgA was detected in controls In this way lysozyme feeding partly substituted for passive transfer of secretory IgA from maternal milk

KEY WORDS Lysozyme immunoglobulins secretory IgA

The difference in incidence of enteric infections between breast fed and bottle fed infants is well known A protective role of breast milk against enteric infections has been suggested from several observations (3 15) The establishment of secretory IgA as the predominant immunoglobulin in milk (10) offered new evidence on the protective role and importance of breast feeding

Another breast milk component—lysozyme—has also been detected in plasma saliva and tears (6 1) This enzyme is known to possess several biological activities closely connected with mechanisms protecting the organism against infection (5) Low levels found in small for date newborns may be partly responsible for their increased susceptibility to infection (11)

In breast milk the level of lysozyme is about 2 mg/ml It modifies the digestive function of breast fed infants influences the character of their faeces and together with Bifidus factor promotes growth of *Lactobacillus bifidus* and inhibits that of *E coli* (5)

Thus bottle fed infants lack two important components of breast milk secretory IgA and lysozyme We have previously demonstrated that oral colonisation with a non enteropathogenic *E coli* strain evoked earlier and higher serum antibody and coproantibody responses as well as secretory IgA production in the intestine of artificially fed infants than in controls (13 14) In the present work we have studied the influence of lysozyme feeding on the production of serum immunoglobulins and secretory IgA in the intestine of artificially fed full term and premature infants as compared with control groups

MATERIALS AND METHODS

All infants under long term investigation were kept in the Department for healthy infants of our Institute from birth until the age of six months Informed consent was obtained from each parent or guardian concerning the

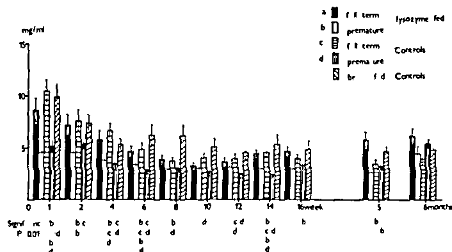


Fig 1 Serum IgG levels in infants

investigation of the infants in accordance with institutional policies A total of 79 infants were divided into five groups 15 full term and 18 premature infants were given egg white lysozyme (10 mg/100 ml of milk formula) from the 1st to the 8th week of age Lysozyme was kindly provided by Società Prodotti Antibiotici Milano Italy (purified crystalline egg white lysozyme in tablets 10 mg each)

13 full term and 13 premature artificially fed infants as well as 20 breast fed infants were followed as controls

Blood and stool samples were taken in the first and second week of life then every fortnight for a period of 16 weeks and finally at ages five and six months All samples were stored on dry ice at −70°C The presence of IgM IgA and IgG in sera and secretory IgA in stool filtrates was first determined qualitatively by a double immunodiffusion technique in a micromodification of the Ouchterlony method using monospecific antisera (IDP set Sevac Prague) The concentration in positive samples was estimated by radial immunodiffusion according to Fahey & Mc Kelvey (4) The coefficient of variance for this method varied between 5 and 8% Lysozyme levels in stool filtrates were estimated by a photometric method (12) Levels of secretory IgA in stool filtrates were statistically evaluated using the chi square test of the 2×2 contingency table For statistical evaluation of serum immunoglobulin levels the one way analysis of variance of log transformed values was used for each interval The mean values of the groups were compared by Scheffe s S method (16) For the SE estimation non transformed data were used

RESULTS

Both groups of full term infants had higher levels of serum IgG than premature infants during the whole period of investigation Serum IgA levels were higher in full term infants from the 12th week there were no dif

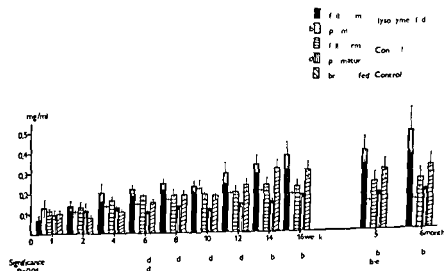


Fig 2 Serum IgM levels in infants

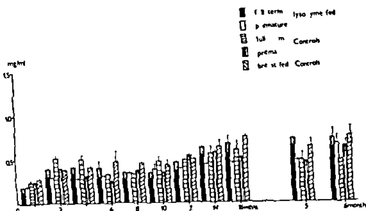


Fig 3 Serum IgA levels in infants

ferences found in IgM levels between full term and premature infants (Figs 1 2 3) Lysozyme administration did not influence serum IgG IgM and IgA levels The influence of lysozyme feeding on the secretory IgA levels in stool filtrites is shown in Table 1 and Fig 4 Lysozyme in stool filtrates from lysozyme fed infants was found only in trace amounts

DISCUSSION

Secretory IgA was influenced by lysozyme feeding in full term artificially fed infants Girard & De Kalbermatten (9) in studies of the antibody activity of human duodenal fluid reported that lysozyme stimulated phago cytosis of IgA and IgM Fubara & Freter (7) suggested a protective effect of secretory IgA which is more resistant to intestinal degrada tion than IgM and IgG derived from serum In the present work secretory IgA was detected in stool filtrates of full term lysozyme fed infants as well as in breast fed ones Fubara & Freter (8) described mechanisms by which intestinal antibodies may be delivered into the lumen of the intestine Their results indicate that an antibody may appear in the intestine as a result of local synthesis or by derivation from a serum antibody Bull et al (2) in a study of human IgA have also shown that IgA from the intestine had properties of 11 S and 7 S IgA On the other hand IgA produced in the intestine was found in the serum In contrast to their findings we could not show an in crease in serum IgA in full term lysozyme fed infants when compared with controls

The explanation of mechanisms of lysozyme

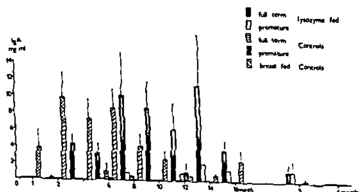
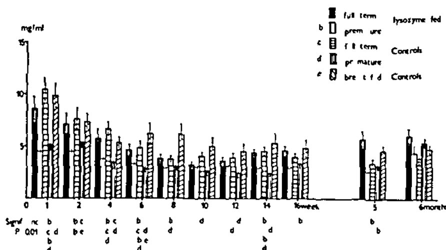


Fig 4 Secretory IgA levels in stool filtrates


Fig 1 Serum IgG levels in infants

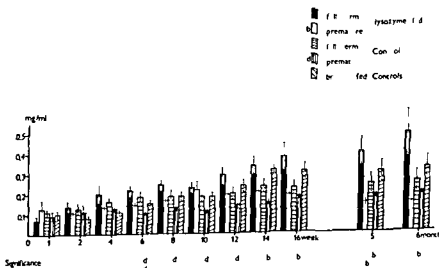
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Fig 2 Serum IgM levels in infants

Acta Pædiatr Scand 66 713–717 1977

URINARY HYPOXANTHINE XANTHINE AND URIC ACID EXCRETION IN NEWBORN INFANTS WITH PERINATAL COMPLICATIONS

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ABSTRACT Manzke H Dorner K and Grunitz J (Department of Paediatrics University Hospital Kiel Federal Republic Germany) Urinary Hypoxanthine Xanthine and Uric Acid Excretion in Newborn Infants with Perinatal Complications Acta Paediatr Scand 66 713 1977.—The concentration of hypoxanthine xanthine and uric acid in the first 24-h urine of 105 newborn infants was measured densitometrically by thin layer chromatography 45 of them had moderate or severe perinatal complications Among these newborns 26 infants with perinatal complications (58%) and 4 infants without perinatal complications (7%) had an elevated urinary excretion rate of hypoxanthine Urinary xanthine was not increased uric acid was slightly higher in the group of infants with perinatal complications It seems that a crucial mark is involved if the rate of hypoxanthine exceeds 15% of the total urinary oxypurine excretion or if related to urinary creatinine more than 0 075 µmol hypoxanthine/µmol creatinine Apparently with hypoxic newborns increased values of urinary hypoxanthine excretion can be used to quantify the lack of oxygen retrospectively

KEY WORDS Newborn infants perinatal complications urinary oxypurine excretion

During the first days of life the catabolism of purines is increased in newborn infants This leads to an overproduction of uric acid (9) in particular in premature infants with respiratory disorders (10) Moreover the reabsorption of uric acid by the renal tubular cells seems to be decreased (13) As a result of reduced fluid intake and diminished fluid excretion uric acid often precipitates in the renal tubuli

In newborn infants dying during the first days of life uric acid infarctions can easily be found by post mortem examination of the kidney The analysis of these deposits in the renal tubuli reveals along with uric acid a high percentage of hypoxanthine and xanthine (7)

Stimulated by the studies of Saugstad (11 12) who was the first to report about elevated hypoxanthine concentrations in the blood of hypoxic newborn infants we examined the urinary oxypurine excretion of infants without perinatal complications and those with moderate and severe perinatal complications The aim of the study is to investigate whether urinary purine metabolites may be used to diagnose hypoxia in newborn infants retrospectively

PATIENTS AND METHODS

All infants studied could be divided into three groups

1 Newborn infants without birth complications=infants born by spontaneous delivery with Apgar scores 9 or 10 one min after birth and without attracting attention during their first day of life

2 Newborn infants with moderate birth complications infants born with Apgar scores 5–8 recovering very quickly (Apgar score>8 five min after birth) and being well during the following time

3 Newborn infants with severe birth (perinatal) complications=infants born with Apgar scores 4–8 one min after birth recovering slowly and showing signs of a latent shock or breathing difficulties during the course of their first day of life

Immediately after birth all infants examined were transferred from the delivery room to the Observation Unit of the University Hospital for Obstetrics or to the Intensive Care Unit of the University Hospital for Children Urine was collected during the first 24 hours of life It was en

Table 1 *Secretory IgA levels in stool filtrates*

Groups of infants	No of infants: Total	No of infants: sIgA positive	No of stool samples: sIgA positive	No of stool samples: sIgA negative	No of stool samples: Total	Significance
A Full term lysozyme fed (artificially fed)	15	10	52	113	165	A C $\chi^2=7.83$ $p<0.01$
B Premature lysozyme fed	18	4	5	175	180	A E not significant
C Full term controls (artificially fed)	13	1	3	127	130	A D not significant
D Premature controls	13	1	2	127	129	A B not significant
E Breast fed controls	20	19	75	112	187	E C $\chi^2=21.63$ $p<0.01$

We considered as positive those infants in whome the sIgA was detected at least once in stool filtrates during the whole period of investigation

action still remains unclear It can be suggested that the lysozyme protein molecules have an antigenic effect The lack of antibody response in serum however supports the idea of a local action Lysozyme might exert an enzymatic lysis of bacterial cell wall and their antigenic structures stimulate a local immune response

The fact that artificially fed infants have no secretory IgA and are deprived of sources from maternal milk stimulated our attempts to humanise artificial feeding

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Submitted Sept 9 1976
Accepted April 27 1977

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reference substance (3 6 8) then hypoxanthine excretion is also higher in infants with birth complications than in those without However the individual values scatter more widely than in the approach underlying Fig 1 A borderline between elevated and non elevated values can be established at 0 075 µmol hypoxanthine/µmol creatinine The mean values ±S D of the urinary hypoxanthine/creatinine excretion for the three groups are as follows infants without birth complications 0 0458±0 0323 infants with moderate birth complications 0 0664±0 0597 infants with severe birth complications 0 1588±0 0882

Due to the wide range of the individual values within the groups a significant difference of the mean values (t test) could only be computed between the infants without birth complications and the infants with severe birth complications ($p<0\ 001$)

Interrelationships between urinary hypoxanthine uric acid creatinine and osmolarity

Fig 2 shows the fairly close relationship between the urinary creatinine and hypoxanthine values ($r=0\ 61$) There was also a positive correlation between the urinary creatinine and osmolarity ($r=0\ 43$ $p<0\ 01$) as well as between the urinary hypoxanthine and osmolarity values ($r=0\ 32$ $p<0\ 05$) However there was neither any significant correlation between the urinary creatinine and the uric acid values ($r=0\ 12$ $p>0\ 4$) nor between the osmolarity and the uric acid values ($r=0\ 07$ $p>0\ 5$) These data are from 78 newborn infants who received only 10–20 ml glucose solution per kg body weight during the first day of life

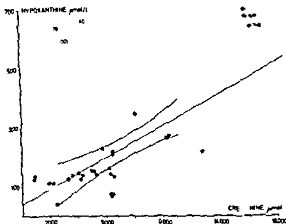


Fig 2 Relationship between urinary hypoxanthine and creatinine excretion in 78 newborn infants during their first day of life

Influence of fluid intake on urinary oxypurine excretion

Next we had to divide our probands into two groups

1 newborn infants who received low fluid supply and

2 newborn infants in particular premature infants who were treated with parenteral infusion of 80–100 ml of glucose solution per kg body weight during the first day of life (and who therefore were passing more urine volume)

Table 1 *Urinary oxypurine excretion as a function of fluid intake in newborn infants during their first day of life*

Urinary output	Newborn infants with low fluid intake (< 0 ml/kg) n = 78	Newborn infants with high fluid intake (>80 ml/kg) n = 3?	t test on mean p
Osmolarity mosmol/l	311±98	187±86	<0 001
Creatinine µmol/l	6 715±4 111	2 160±1 751	<0 001
Total oxypurine µmol/l	2 067±671	2 274±680	>0 1
Hypoxanthine µmol/l	218±181	170±176	<0 001
Uric acid µmol/l	1 800±614	? 116±707	>0 1
Hypoxanthine/creatinine µmol/µmol	0 03 ±0 044	0 056±0 072	<0 01
Uric acid/creatinine µmol/µmol	0 ?68±0 149	0 980±0 404	<0 001

sured that urine collection began no later than 1/2 h after birth

Oxypurine determinations as carried out in this study are based on a modified method reported in a previous paper (2) Evaluations of the thin layer chromatograms were done with a Vitatron TLD densitometer and its integrating recorder UR 402 Hypoxanthine was measured at 254 nm xanthine at 280 nm and uric acid at 293 nm No other oxypurine compounds covered the spots of hypoxanthine xanthine and uric acid as could be shown by treatment of urine samples with xanthinoxydase and uricase Only in some urines a trace of an uv absorbing substance (inosine?) remained within the area of the hypoxanthine The coefficient of variation for the three oxypurines was 5 7% 5 4% and 1 5% (n=12) respectively The addition of 18 2 33 3 and 46 1 ng hypoxanthine or uric acid to 1 ml urine revealed recoveries of 99% 87% and 108% resp 111% 97% and 91% Parallel uric acid determinations carried out with both the densitometric as well as the enzymatic method (Urica quant Biochemica Test Combination Boehringer Mannheim) produced quite similar results (r=0 79 n=42)

The creatinine content of the urine was determined by an autoanalyser method (is yet unpublished) which utilizes dinitroebenzoic acid as the reagent Osmolarity measurements were performed with an osmometer (Knauer Berlin) All analyses were done in duplicate

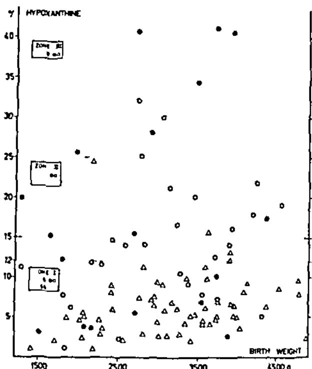


Fig 1 Hypoxanthine rate in the total urinary oxypurine excretion of 105 newborn infants during their first day of life ● infants with severe birth complications ○ infants with moderate birth complications △ infants without birth complications

RESULTS

Proportionality of the urinary oxypurine rates

Urine samples were examined from 105 newborn infants 45 of them had moderate or severe birth complications Fig 1 plots the values of the hypoxanthine rate within the total urinary oxypurine output of all infants As it was difficult to collect the complete 24 h amount of urine the actual amounts of excreted oxypurines could not be determined but only the concentrations within the samples As Fig 1 illustrates the hypoxanthine rates were usually higher in the infants with severe and moderate birth complications than in the newborns without birth complications

In zone I i e with hypoxanthine rates below 12% 19 infants had severe or moderate birth complications and 56 infants had no birth complications

In zone II representing hypoxanthine rates between 12–25% 17 of the 21 infants had birth complications

In zone III representing hypoxanthine rates above 25% all infants had severe or moderate birth complications

Among the infants without birth complications there were only very few that had elevated urinary hypoxanthine rates However a large number of infants with moderate or severe birth complications namely 45% presented normal hypoxanthine rates below 12% There may be two explanations (*a*) the hypoxic state was too short to cause any increased urinary hypoxanthine excretion or (*b*) the 24 h collection time was too long to get information about the hypoxic episode

Furthermore it is unlikely that reduced urine volumes should provide an explanation because the renal clearance of hypoxanthine is much higher than that of uric acid in adults for instance ten times as high (4) In Fig 1 the dividing line for the elevated vs the non elevated hypoxanthine rate within the total urinary oxypurine rate has arbitrarily been set at 15%

If alternatively creatinine is used as the

reference substance (3 6 8) then hypoxanthine excretion is also higher in infants with birth complications than in those without However the individual values scatter more widely than in the approach underlying Fig 1 A borderline between elevated and non elevated values can be established at 0 075 µmol hypoxanthine/µmol creatinine The mean values ±S D of the urinary hypoxanthine/creatinine excretion for the three groups are as follows infants without birth complications 0 0458±0 0323 infants with moderate birth complications 0 0664±0 0597 infants with severe birth complications 0 1588±0 0882

Due to the wide range of the individual values within the groups a significant difference of the mean values (*t* test) could only be computed between the infants without birth complications and the infants with severe birth complications ($p<0.001$)

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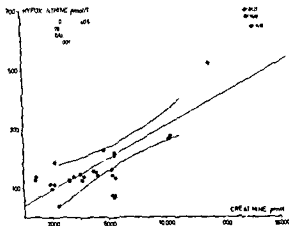


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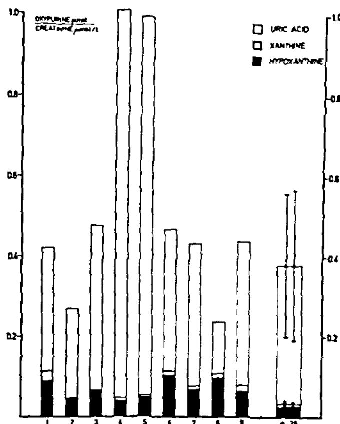


Fig 3 Urinary oxypurine excretion (μmol/μmol creatinine) in 9 newborn infants with severe birth complications during their first day

Table 1 summarizes these two groups the urinary osmolarity was nearly twice as high in the infants with low fluid intake than in the infants with high fluid intake and the creatinine concentration was three times as high in the former than in the latter The total urinary oxypurine concentration however was nearly the same in both groups Correspondingly the hypoxanthine excretion as related to creatinine was approximately a third and the uric acid excretion as related to creatinine 3–4 times lower in infants with low fluid intake than in infants with high fluid intake

Urinary oxypurine excretion and severity of birth complications

The histogram (Fig 3) illustrates the urinary oxypurine excretion as relating to the creatinine in 9 newborn infants with severe birth complications The thin columns represent the individual cases The numerical order of the columns corresponds to the severity of the birth complications the first case being the most severe one All infants had Apgar scores between 4 and 8 one min after birth furthermore complications such as bradycardia of long duration due to coiling of the cord were taken into account In comparison with the control group of 28 newborn infants without birth complications (plotted as a broad column) the hypoxanthine excretion was more than twice their mean value ($\bar{x}$=0 0735±0 0203 resp $\bar{x}$=0 0236±0 0100 μmol/μmol creatinine p<0 001) The excretion of xanthine varies considerably In some infants no xanthine was detectable in their urine The uric acid excretion varies considerably too and no statistical significance could be computed between the two groups ($\bar{x}$=0 4358±0 2712 resp $\bar{x}$=0 3491 ±0 1829 μmol/μmol creatinine p>0 4) There is also no significant difference between the mean values of the total urinary oxypurine excretion between the infants with birth complications and the control group ($\bar{x}$=0 5353± 0 3002 resp $\bar{x}$=0 3800±0 1927 μmol/μmol creatinine p<0 1) All infants compared here received only low fluid supply to exclude the influence of different amounts of fluid intake on the urinary oxypurine excretion

DISCUSSION

Hypoxanthine xanthine and uric acid represent the endproducts of the purine metabolism Under normal conditions more than 90% of the urinary oxypurines are excreted as uric acid (1) and less than 10% as hypoxanthine and xanthine (Fig 4) In newborn infants the hypoxanthine rate amounts to as much as 12% physiologically It seems that a crucial mark is reached if the rate of hypoxanthine exceeds 15% of the total urinary oxypurine excretion or if related to urinary creatinine more than 0 075 μmol hypoxanthine/μmol creatinine Our results confirm indirectly those reported by Saugstad (11 12) who found increased levels of hypoxanthine in the blood of hypoxic newborns

On the other hand clinical experiments with allopurinol an inhibitor of the enzyme xan

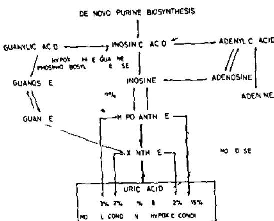


Fig 4 Pathways in purine metabolism and salvage (according to 14)

thinoxydase show that the higher the hy poxanthine concentration in the blood the more hypoxanthine can be recycled to inosinic acid through the action of the enzyme hypo xanthine guanine phosphoribosyl transferase This is called the salvage pathway (14) It may explain why the total urinary oxypurine excre tion in hypoxic newborns is not significantly higher than in normoxic newborns Further more a decreased blood perfusion rate in the kidneys could cause a temporary decrease in the clearance of oxypurines in hypoxic new borns The amount of fluid intake is central for an adequate renal oxypurine excretion dur ing the first days of life (7 10) In utero a good clearance of the placenta for hypoxanthine can be inferred from the positive arterio venous difference in cord blood (5)

After birth renal clearance of hypoxanthine approaches that of creatinine whereas the renal clearance of uric acid is much less (4) In newborn infants with hypoxia of short dur ı tion no significantly elevated urinary oxy purine rates will be found if the urine is col lected during too a long period Otherwise in creased urinary hypoxanthine concentration in newborn infants if measured in the first post natal urine may derive from maternal blood via placenta transfer (5)

Each of these variables must be taken into account if increased values of urinary hy poxanthine excretion are used to quantify the lack of oxygen in hypoxic newborn infants retrospectively

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Submitted Sept 3 1976
Accepted April 15 1977

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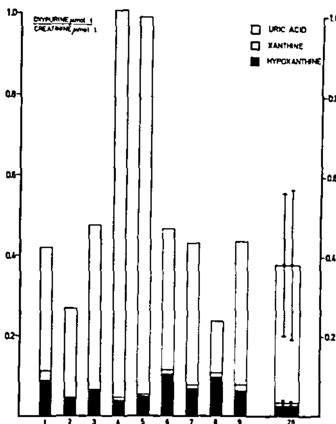


Fig 3 Urinary oxypurine excretion (µmol/µmol creatinine) in 9 newborn infants with severe birth complications during their first day

Table 1 summarizes these two groups the urinary osmolarity was nearly twice as high in the infants with low fluid intake than in the infants with high fluid intake and the creatinine concentration was three times as high in the former than in the latter The total urinary oxypurine concentration however was nearly the same in both groups Correspondingly the hypoxanthine excretion as related to creatinine was approximately a third and the uric acid excretion as related to creatinine 3–4 times lower in infants with low fluid intake than in infants with high fluid intake

Urinary oxypurine excretion and severity of birth complications

The histogram (Fig 3) illustrates the urinary oxypurine excretion as relating to the creatinine in 9 newborn infants with severe birth complications The thin columns represent the individual cases The numerical order of the columns corresponds to the severity of the birth complications the first case being the most severe one All infants had Apgar scores between 4 and 8 one min after birth further more complications such as bradycardia of long duration due to coiling of the cord were taken into account In comparison with the control group of 28 newborn infants without birth complications (plotted as a broad column) the hypoxanthine excretion was more than twice their mean value ($\bar{x}$=0 0735±0 0203 resp $\bar{x}$=0 0236±0 0100 µmol/µmol creatinine $p<0\ 001$) The excretion of xanthine varies considerably In some infants no xanthine was detectable in their urine The uric acid excretion varies considerably too and no statistical significance could be computed between the two groups ($\bar{x}$=0 4358±0 2712 resp $\bar{x}$=0 3491 ±0 1829 µmol/µmol creatinine $p>0\ 4$) There is also no significant difference between the mean values of the total urinary oxypurine excretion between the infants with birth complications and the control group ($\bar{x}$=0 5353± 0 3002 resp $\bar{x}$=0 3800±0 1927 µmol/µmol creatinine $p<0\ 1$) All infants compared here received only low fluid supply to exclude the influence of different amounts of fluid intake on the urinary oxypurine excretion

DISCUSSION

Hypoxanthine xanthine and uric acid represent the endproducts of the purine metabolism Under normal conditions more than 90% of the urinary oxypurines are excreted as uric acid (1) and less than 10% as hypoxanthine and xanthine (Fig 4) In newborn infants the hypoxanthine rate amounts to as much as 12% physiologically It seems that a crucial mark is reached if the rate of hypoxanthine exceeds 15% of the total urinary oxypurine excretion or if related to urinary creatinine more than 0 075 µmol hypoxanthine/µmol creatinine Our results confirm indirectly those reported by Saugstad (11 12) who found increased levels of hypoxanthine in the blood of hypoxic newborns

On the other hand clinical experiments with allopurinol an inhibitor of the enzyme xan

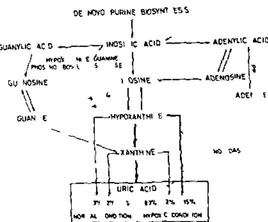


Fig 4 Pathways in purine metabolism and salvage (according to 14)

thinoxydase show that the higher the hypoxanthine concentration in the blood the more hypoxanthine can be recycled to inosinic acid through the action of the enzyme hypoxanthine guanine phosphoribosyl transferase This is called the salvage pathway (14) It may explain why the total urinary oxypurine excretion in hypoxic newborns is not significantly higher than in normoxic newborns Further more a decreased blood perfusion rate in the kidneys could cause a temporary decrease in the clearance of oxypurines in hypoxic newborns The amount of fluid intake is central for an adequate renal oxypurine excretion during the first days of life (7 10) *In utero* a good clearance of the placenta for hypoxanthine can be inferred from the positive arterio venous difference in cord blood (5)

After birth renal clearance of hypoxanthine approaches that of creatinine whereas the renal clearance of uric acid is much less (4) In newborn infants with hypoxia of short duration no significantly elevated urinary oxypurine rates will be found if the urine is collected during too a long period Otherwise increased urinary hypoxanthine concentration in newborn infants if measured in the first postnatal urine may derive from maternal blood via placenta transfer (5)

Each of these variables must be taken into account if increased values of urinary hypoxanthine excretion are used to quantify the lack of oxygen in hypoxic newborn infants retrospectively

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Submitted Sept 3 1976
Accepted April 15 1977

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Acta Pædiatr Scand 66 719–722 1977

IRON ABSORPTION FROM INFANT MILK FORMULA AND THE OPTIMAL LEVEL OF IRON SUPPLEMENTATION

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ABSTRACT Saarinen U M and Siimes M A (Children s Hospital University of Helsinki Finland) Iron absorption from infant milk formula and the optimal level of iron supplementation Acta Paediatr Scand 66 719 1977.—Thirty healthy infants aged 11–13 months were studied with regard to the iron absorption from proprietary milk formula The infants were divided into three groups (I–III) depending on the concentration of iron in the formula 0 8 (I) 6 8 (II) and 12 8 (III) mg/l respectively The calculated amount of iron absorbed per test dose of 50 ml of milk averaged 5 μg (I) 32 μg (II) and 43 μg (III) Group I differed significantly from groups II and III No correlation was found between iron absorption and hemoglobin MCV serum transferrin saturation or serum ferritin within the range of normal values Our findings suggest that at least 7 mg of iron as ferrous sulphate per litre of formula is required to prevent iron deficiency

KEY WORDS Infant nutrition iron absorption milk

Iron deficiency in infancy remains a common nutritional problem One of the most effective and widespread methods of preventing this deficiency is the use of iron fortified formula Most proprietary formulas contain 10 to 12 mg of iron per litre in the form of ferrous sulphate and this level of fortification is found to be effective in the prevention of the iron deficiency that commonly develops when neonatal iron stores are exhausted after 2 months of age in premature infants and after 4 months of age in term infants (1 2) However a number of recent studies have indicated that even modest degree of iron excess could be harmful to the infant (6 8 17) These considerations raise the question of whether present levels of iron in fortified formula are optimal or whether a smaller amount would be just as effective A salient feature of iron absorption is that the percentage that is absorbed decreases as the dose is increased (4) At the present level of iron fortification of 10 to 12 mg/l an average of about 4% of the iron or 0 4 to 0 5 mg/l is absorbed (14)

This study attempts to find a level of iron supplementation for infant milk formula that would achieve sufficient iron absorption to prevent iron deficiency without unnecessarily high supplementation

MATERIALS AND METHODS

Our study population consisted of 30 infants aged 11 2 to 13 2 months The infants were selected from a group of 250 healthy infants who were followed from birth to one year of age at frequent intervals for dietary instructions and blood sampling In all infants solid foods were introduced late cooked vegetables at 3 5 months cereals at 5 months and meat and eggs at 6 months of age All were initially breast fed and all were weaned prior to the age of 5 months 6 of them to home prepared cow s milk formula which was changed to commercial fresh cow s milk at 6 months of age and 24 of them to an iron supplemented (11 mg Fe/l) proprietary infant milk formula

Iron nutrition was evaluated by the use of hemoglobin RBC indices (a Model S Coulter Counter) serum transferrin saturation (5 13) and serum ferritin (15) (Table I) Because depleted iron stores should markedly influence iron absorption (10 16) two infants with minimal or ex

Table 1 *The infant material*

The means and the ranges of the values are shown No statistical differences were found between the groups

	I (n=10)	II (n=9)	III (n=10)
Weight (kg)	10 0 (9 0–11 1)	9 8 (9 0–11 5)	10 0 (8 1–11 5)
Length (cm)	76 9 (74 6–79 3)	77 0 (74 4–79 3)	76 5 (72 6–80 4)
Hb (g/100 ml)	12 6 (11 3–13 8)	12 8 (11 7–13 6)	12 4 (11 1–13 7)
MCV (fl)	76 (71–79)	78 (75–81)	77 (72–83)
Transferrin saturation (%)	22 8 (4 4–35 0)	24 4 (11 4–40 7)	27 9 (9 7–60 2)
Ferritin (μg/l)	32 (16–66)	31 (18–43)	30 (19–70)

Geometric mean and the range of the values

hausted storage iron (serum ferritin ≤10 μg/l) were evaluated separately and excluded from the remaining data

Iron absorption studies were performed using radiochemically pure[59] Fe labelled ferrous sulphate (20 mCi/mg New England Nuclear Corporation Boston USA) Precounted doses of 0 8 to 1 0 μCi were mixed with carrier ferrous sulphate in freshly prepared aqueous solution and added to 50 ml of infant milk formula (Tutteli* Valio Oy Helsinki Finland) This milk was given to the infant after 2 hours of fasting followed by an additional hour of fasting Each bottle together with any remaining milk (always less than 5 ml) was counted in order to accurately calculate the ingested volume of formula and dose of isotope The proportion of administered dose retained at 14 days was measured by a whole body counter with four moving detectors at 90° angles

The infants were divided into three study groups each of which received a different concentration of iron in the test milk formula as follows

Group I 0 8 mg/l no ferrous sulphate added

Group II 6 8 mg/l 6 mg of iron as ferrous sulphate added per litre

Group III 12 8 mg/l 12 mg of iron as ferrous sulphate added per litre

All parents were well informed regarding the study and the use of radioactive isotope and participated on a voluntary basis Although we were concerned about the small potential hazard of any use of isotope in infants we felt it could be justified on a one time research basis and using a small number of infants The retained dose of isotope was well within the Scandinavian recommendations on the research use of radioactivity in children namely of magnitude of one year s exposure from natural radiation (12)

RESULTS

The average iron absorption from milk formula was 12% in group I 9% in group II and 7% in group III (Fig 1) The difference between the groups did not reach statistical significance ($p<0\ 10$ between groups I and III) because of the wide range of the values

The calculated amount of iron absorbed per test dose of 50 ml of milk formula averaged 5 μg in group I 32 μg in group II and 43 μg in group III Group I differed significantly from groups II and III ($p<0\ 05$ $p<0\ 001$ respectively) *Although the total quantity of iron absorbed rises with increasing iron concentration* the difference between groups II and III was small and statistically nonsignificant

There was considerable individual variation in iron absorption in each group which did not correlate with the hemoglobin MCV or serum transferrin saturation Surprisingly there was no significant correlation between iron absorption and serum ferritin values within the range of normal serum ferritin values However there was increased absorption in the two infants who were excluded on

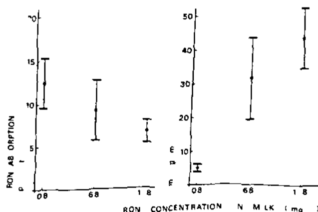


Fig 1 Iron absorption from 50 ml of infant milk formula in % (left) an in μg (right) is correlated with iron concentration in milk The means ± S E M are shown Only the 28 infants with serum ferritin values above 10 μg/l are included

the basis of exhausted iron stores as indicated by serum ferritin ≤10 μg/l The absorption was 49% in one infant in group II and 14% in the other infant in group III an increase of about 5 fold and 2 fold compared to the means of their respective groups

DISCUSSION

Our results indicate that infant milk formula without iron fortification does not meet the requirements of sufficient iron intake (1) On the other hand from our study and from several others it is evident that iron in the form of ferrous sulphate added to infant formulas is absorbed in substantial amounts and is effective in preventing iron deficiency (3 11)

Raising the iron concentration of formula above certain levels results in only minor increases in the total amount of iron absorbed because of a decrease in the percentage absorbed This dose relationship is in accord with previous studies (10) Our findings suggest that at least 7 mg of iron as ferrous sulphate per litre of formula is required to prevent iron deficiency Other data such as those of Gorton & Cross (9) also tend to support this view

It is desirable to use no more fortification than is necessary to prevent iron deficiency The two iron binding proteins of milk lactoferrin and transferrin are normally one third saturated with iron In this condition they show bacteriostatic properties *in vitro* that are lost when the proteins become saturated with iron In milk formulas supplemented with iron these proteins should theoretically become saturated and lose their bacteriostatic properties (6) This might favor the development of enteric infections and alterations in bacterial flora Furthermore iron supplementation in small preterm infants is associated with mild hemolytic states especially in connection with vitamin E deficiency and formulas rich in poly unsaturated fatty acids (8 17) Thus unnecessarily generous iron supplementation should be avoided

ACKNOWLEDGEMENTS

Supported by grants from Foundation for Pediatric Research Finland and Foundation for Chemical Research Finland

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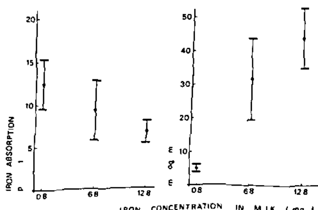


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Acta Pædiatr Scand 66 723-728 1977

PATIENTS AT A PAEDIATRIC OUT PATIENT CLINIC

A Study with Particular Reference to Psychological and Social Background Factors I The Problem Material Methods and the Actual Visit

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ABSTRACT Jonsell R (Department of Paediatrics University Hospital Umeå and Department of Child and Youth Psychiatry Karolinska Institutet S t Coran s Children s Hospital Stockholm Sweden) Patients at a paediatric out patient clinic A study with particular reference to psychological and social background factors I Acta Paediatr Scand 66 723 1977 —A large proportion of the children who attend paediatric clinics present with indefinite somatic symptoms or complaints which are clearly or possibly of a psychological nature At the same time it is known that patients with serious psychological problems tend to seek help far too late This study is an attempt to find out whether the patients who are liable to become problem cases in the long term can be identified by the paediatrician at an early stage and if so in what ways these patients differ from others in a paediatric clientele

All the patients who attended the Paediatric Out Patient Clinic at Umeå Hospital during the course of one year were assessed by the physicians there It was judged that psychological factors of importance were involved in 5 2% that the importance was doubtful in 8 3% and that no psychological factors were involved in 86 5% The proportion of cases involving psychological factors of importance increased with age and amounted to 17% among the 10 to 15 year olds

KEY WORDS Children out patient symptoms psychological

Psychological symptoms and social background are important factors behind the admission of children to hospital (5 29 36) It has also been shown that paediatric wards and out patient clinics offer a large field of work for child psychiatrists (10 11 20 35) The panorama of symptoms which may have a psychological background that the somatic physician often overlooks has been excellently reviewed by Apley & McKeith (4) and described by other authors (1 27 38)

The problem

The number of young people with serious psychological complaints and social maladjustment has become a major problem for the community in Sweden and for many other countries Psychiatrists dealing with children adolescents and adults often meet these patients far too late when their situation has become difficult to influence

Paediatricians and other physicians see many children—as out patients or hospital cases—with symptoms (e g abdominal pains headache poor eating) for which no organic explanation can be found

Main questions initiating this study

If some of the patients who are brought to a paediatrician with symptoms that lack a somatic background are identical with those who much later pose intractable problems for psychiatrists and social authorities? If so can an early diagnosis and adequate measures by

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This and a following paper (17) constitute a short summary of a thesis in Swedish (16) from which more detailed information can be obtained (the summary tables and figures are in English)

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17 Williams M L Shott R J O Neal P L & Oski F A Role of dietary iron and fat on vitamin E deficiency anemia of infancy *N Engl J Med 292* 887 1975

Submitted Jan 12 1977
Accepted May 3 1977

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Acta Pædiatr Scand 66 723–728 1977

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in the tables apply $p \leq 0\ 001$ $p \leq 0\ 01$ $p \leq 0\ 05$ and n s $p > 0\ 05$ where p is the probability of an erroneusly rejected null hypothesis

A note was made from the case records of whether the visit was by appointment the origin of any referral the physicians s measures as well as the diagnosis of symptoms with a four digit ICD code occasionally supplemented in order to describe some symptoms and reasons for a paediatric examination that were not covered by the code Complaints where a psychological cause was probable or suspected were furthermore coded in accordance with a separate list the assessment of what to include being based on the examining physician s opinion as indicated by the case report

RESULTS

The presence of psychological factors at the actual visit was judged by the examining physician as shown in Table 1 The distribution by age is reproduced in Fig 1 The composition of the two groups of subjects and the control group is given in Table 2 A complete set of results has been calculated for the PD group but as they lie in between the results for groups PY and PN they have not been included in the following account About two-thirds of the patients came to the clinic without being referred this was particularly common amongst the youngest children Visits by appointment were 67 2% in PY and 45 8% in PN ($p < 0\ 001$)

The most common diagnoses and symptoms are listed in Table 3 For the most common complaints where a psychological factor was probable or suspected a breakdown by age is given in Fig 2

Table 2 *Composition of subject and control groups*

Age years completed	PF group		
	PY N=198 (%)	PD N=300 (%)	PN N=544 (%)
0– 3	23 3	36 7	29 8
4– 9	39 4	41 3	39 7
10–15	37 3	27 0	30 5
Boys	57 6	49 3	53 3
Resident in Umeå + 40 km radius	8 8	85 7	83 5

Table 3 *Actual visit*

Percentage with the diagnosis/symptom in each PF group

Diagnoses/symptoms	PY N=198	PN N=544	Difference PY–PN
Mental and nervous disorders and symptoms	27 3	–	
Behaviour disorders	17 2	1 8	
Functional symptoms	12 1	4 2	
Abdominal pains	24 2	2 0	
Headache	6 1	3 7	n s
Enuresis	12 1	2 4	
Gastroenteritis acuta	3 5	5 5	n s
Upper respiratory infections	15 2	32 0	
Asthma and bronchitis asthmatica	3 0	10 7	
Infections of the genito-urinary system	3 5	7 7	n s
Dermatological diseases	1 5	6 4	*
Diffuse reasons for attendance	6 6	4 0	n s

If a patient had several diagnoses they were all registered If two or more belonged to the same diagnostic group they have been included as a single diagnosis in the table

In some instances it was only after several contacts with the health service that the background to the patient s symptoms became clear The following case report provides an illustration of this

Girl 4 years PD 112

Previous history Two suspected urinary tract infections treated with sulphonamides at another centre

Three weeks before the actual visit emergency visit because of abdominal pains for the previous 24 hours Assessed as constipation given laxatives Urine culture negative Advised to return if symptoms continued

Actual visit Emergency case in the evening Had stayed with relatives for two weeks but on the day of the current visit had returned to her mother where she started to complain of stomach pains urinated frequently and complained that this was painful The pains had ceased when she arrived at the clinic She was found to be alert and unaffected Urine culture negative but in view of the previous history it was planned to admit her for an examination of the urinary tract The mother s history gave no indication of a strained social situation

Subsequent history The patient was admitted as an emergency case two days later because of recurrence of the abdominal pains together with pains on defaecation Nothing abnormal was found at urography and micturition urethrocystography Laboratory tests were normal During her stay on the ward it became clear that her parents had separated six months earlier The father had asked for a divorce while the mother denied any marital discord As the mother worked in the evenings it was considered possible that the girl was insecure in her care An appointment was made to interview both parents

Table 1 *Examining physician s assessment of whether psychological factors were involved at initial visit*

PF	n	%
Yes (PY)	206	5 2
Doubtful (PD)	331	8 3
No (PN)	3 460	86 5
Total	3 997	100 0

the paediatrician prevent a serious psychiatric or social maldevelopment in certain cases?

Specific questions in this study

1 Can a paediatrician without specialist training in child psychiatry pick out when requested to do so the patients who have non somatic problems that are conditioned by psychological and social conditions?

2 Have these patients previously had particularly frequent contacts with paediatric services in general or for psychological problems in particular and have they been in touch with child psychiatrists?

3 Does the composition of the family or its social and economic conditions contain factors which could involve insecurity for the child? Has the family been in particular need of support from social authorities?

4 Have the parents a high morbidity in general or in particular for certain types of complaints e g mental nervous disorders?

MATERIAL AND METHODS

The material was collected at the paediatric out patient clinic of Umeå in northern Sweden This was the only clinic staffed with paediatricians in a large area with a maximal distance of about 400 km to the clinic For a large part of the population however Umeå was quite close 18 399 *children lived within 40 km of the town* centre and of the other 16 180 children aged 0–15 years the majority had their home within 120 km (26) Families with children with a very low economic standard or poor housing were uncommon (26 31) With regards to the public system for social security in Sweden then economic factors cannot be considered to have had a selective effect upon the persons who attended the paediatric clinic

A referral from a physician was not required in order to attend the paediatric clinic a facility which was utilized above all by parents living in and close to the town In view of the absence of paediatric resources outside the clinic it can be assumed that most cases presenting substantial paediatric problems were remitted or came straight to the paediatric clinic

In the year from 1 July 1970 to 30 June 1971 a record was made of all visits to paediatricians at the Umeå Hospital Patients making an initial visit (defined as one which was not a follow up visit) numbered 4 152 of whom 37 6% had an appointment 34 9% came as emergency cases on Monday–Friday from 8 a m –5 p m and 27 5% were emergency cases outside these hours

Of all the initial visists 86 2% were received by twelve physicians of whom two *had more than 6 years paediatric* experience seven had 3–5 years and three had 1–3 years six of them had at least 6 months experience of child psychiatry The material for the present study was limited to initial visits by children under 16 years of age whose permanent adress lay within the area served by the hospital These limitations left 3 997 initial visits of which 52 9% were boys and 86 9% were living within 40 km from Umeå Distribution by age is shown in Fig 1

At each visit the examining physician judged whether psychological factors had an essential bearing on the child s symptoms using the three alternatives Yes Doubtful and No The term psychological factor is used here in a wide sense i e the fundamental cause might be the child s constitution emotional or social environment or endogenous psychological disorders Two groups of subjects were formed from all the cases which were judged as 1) Psychological Factor Yes (PY) and 2) Psychological Factor Doubtful (PD) In addition a control group was obtained from a sample of the Psychological Factor No (PN) cases matched for the variables sex age and place of residence

Statistical methods

Chi square analyses (in 2×2 contingency tables with Yates correction) mean and median tests and product moment correlations were used (13 14 32 33) The following levels of significance and corresponding symbols

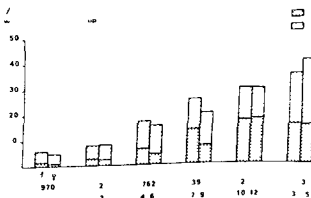


Fig 1 Basic material Proportion of PY and PD cases among the total number of initial visits according to sex and age group Age in completed years

in the tables apply $p \leq 0\ 001$ $p \leq 0\ 01$ $p \leq 0\ 05$ and n s $p > 0\ 05$ where p is the probability of an erroneusly rejected null hypothesis

A note was made from the case records of whether the visit was by appointment the origin of any referral the physicians s measures as well as the diagnosis of symptoms with a four digit ICD code occasionally supplemented in order to describe some symptoms and reasons for a paediatric examination that were not covered by the code Complaints where a psychological cause was probable or suspected were furthermore coded in accordance with a separate list the assessment of what to include being based on the examining physician s opinion as indicated by the case report

RESULTS

The presence of psychological factors at the actual visit was judged by the examining physician as shown in Table 1 The distribution by age is reproduced in Fig 1 The composition of the two groups of subjects and the control group is given in Table 2 A complete set of results has been calculated for the PD group but as they lie in between the results for groups PY and PN they have not been included in the following account About two-thirds of the patients came to the clinic with out being referred this was particularly common amongst the youngest children Visits by appointment were 67 2% in PY and 45 8% in PN ($p < 0\ 001$)

The most common diagnoses and symptoms are listed in Table 3 For the most common complaints where a psychological factor was probable or suspected a breakdown by age is given in Fig 2

Table 2 *Composition of subject and control groups*

Age years completed	PF group PY $N=198$ (%)	PD $N=300$ (%)	PN $N=544$ (%)
0– 3	23 3	36 7	29 8
4– 9	39 4	41 3	39 7
10–15	37 3	22 0	30 5
Boys	57 6	49 3	53 3
Resident in Umeå + 40 km radius	82 8	85 7	83 5

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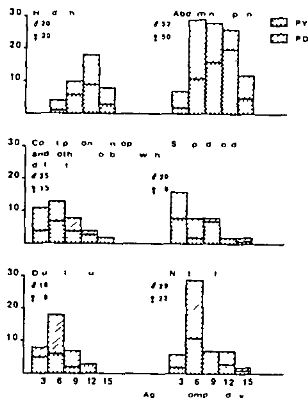


Fig 2 Actual contact Distribution of some common psychogenic complaints (suspected and probable) in different age groups

Other sources The mother had given birth to her first child at the age of 17 This child had been fathered by another man Since that pregnancy the mother had experienced severals periods of back trouble gastritis abdominal pains and psychoneurosis for which she had been registered sick

In 19 7% of the PY group the patients either received a referral to the child psychiatry clinic or were recommended to renew an existing contact or to make use of a referral which had been issued earlier Many parents either declined a proposed referral straight away or else let it lapse even though an appointment had been arranged within a week (Table 4) In such cases the paediatrician often seemed to believe that the patient was being looked after and it was therefore no form of follow up arranged at the paediatric clinic

Cases which were neither followed up at the paediatric clinic nor attended the child psychiatry clinic within two years accounted for 36 3% of the PY group and 43 7% of the PN group (n s)

DISCUSSION

Comparisons with other paediatric clinics are complicated by the lack of detailed statistics for out patient care The predominance of boys and very young children agrees with findings from other studies in the USA and Sweden (7 9 37)

In two separate studies motivated by particular interest in the subject Norstedt (23) (paediatrician in a Stockholm suburb) and Bergfors (6) (paediatric clinic of Skellefteå in northern Sweden) found a proportion of patients attending with psychological and psychosomatic problems at their outpatient clinics that were somewhat higher than the proportion of PY cases in the present study The distribution by age for certain psychogenic complaints in this report is in line with other reports (8 23 39)

A considerable proportion of the patients in the present study presented indefinite somatic symptoms and the frequency of both respiratory infections and other virus infections was relatively high even in the PY group This may reflect a reduced tolerance to the child s symptoms by parents who are under stress for other reasons (21 28) It is also conceivable that the somatic symptoms are used more or less unconsciously as a passport for discussing quite different problems with a physician (22

Table 4 *Number of PY cases where contact with the child psychiatry clinic was proposed or a referral made at the actual contact and subsequent non attendance at the clinic*

	Total (*n*)	Did not attend the child psychiatry clinic within two years (%)
Referral made or contact established in another way	39	25 6
Referral/contact proposed but parents declined	7	–
Paediatrician considered that contact with or referral to child psychiatry clinic was indicated	46	36 9

34 37) Many of the PY cases were left without an active paediatric follow up or a referral elsewhere Other studies have shown that patients who are most in need of help do not return if the initiative for this is left to the parents and the patient (2 19) Since very few parents refuse measures and referral when their children are ill it is surprising how many refuse a referral to a child psychiatrist This attitude which is often reinforced by relatives neighbours etc probably rests on preconceptions about child psychiatry and the concept of psychiatric disorder This fact was indicated in other studies by interviews with parents (12 18 25)

Several studies suggest that many of the patients who are not followed up return to health services later on with the same or other indefinite symptoms (3 15 24 30)

Previous contacts with the health service certain background data and a comprehensive discussion are presented in a second paper (17)

ACKNOWLEDGEMENTS

Financial support to this study was obtained from the Bank of Sweden Tercentenary Fund (project 77/69) and University of Umeå (Ungraduated Researchers Fund)

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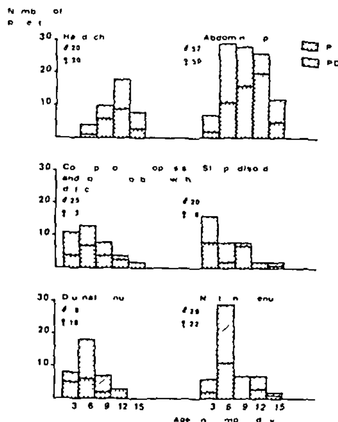


Fig. 2 Actual contact. Distribution of some common psychogenic complaints (suspected and probable) in different age groups.

Other sources. The mother had given birth to her first child at the age of 17. This child had been fathered by another man. Since that pregnancy the mother had experienced severals periods of back trouble, gastritis, abdominal pains and psychoneurosis for which she had been registered sick.

In 19.7% of the PY group the patients either received a referral to the child psychiatry clinic or were recommended to renew an existing contact or to make use of a referral which had been issued earlier. Many parents either declined a proposed referral straight away or else let it lapse, even though an appointment had been arranged within a week (Table 4). In such cases the paediatrician often seemed to believe that the patient was being looked after and it was therefore no form of follow up arranged at the paediatric clinic.

Cases which were neither followed up at the paediatric clinic nor attended the child psychiatry clinic within two years accounted for 36.3% of the PY group and 43.7% of the PN group (n.s.).

DISCUSSION

Comparisons with other paediatric clinics are complicated by the lack of detailed statistics for out-patient care. The predominance of boys and very young children agrees with findings from other studies in the USA and Sweden (7, 9, 37).

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A considerable proportion of the patients in the present study presented indefinite somatic symptoms and the frequency of both respiratory infections and other virus infections was relatively high even in the PY group. This may reflect a reduced tolerance to the child's symptoms by parents who are under stress for other reasons (21, 28). It is also conceivable that the somatic symptoms are used more or less unconsciously as a "passport" for discussing quite different problems with a physician (22

Table 4. *Number of PY cases where contact with the child psychiatry clinic was proposed or a referral made at the actual contact and subsequent non-attendance at the clinic*

	Total (*n*)	Did not attend the child psychiatry clinic within two years (%)
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Referral/contact proposed but parents declined	7	–
Paediatrician considered that contact with or referral to child psychiatry clinic was indicated	46	36.9

Acta Pædiatr Scand 66 729–734 1977

PATIENTS AT A PAEDIATRIC OUT PATIENT CLINIC

A Study with Particular Reference to Psychological and Social Background Factors
II Earlier Contacts with the Health Service Social Background Parents Morbidity General Discussion and Recommendations

R JONSELL

From the Department of Paediatrics University Hospital Umeå and the Department of Child and Youth Psychiatry Karolinska Institutet S t Goran s Children s Hospital Stockholm Sweden

ABSTRACT Jonsell R (Department of Paediatrics University Hospital Umeå and Department of Child and Youth Psychiatry S t Goran s Children s Hospital Stockholm Sweden) Patients at a paediatric out patient clinic A study with particular reference to psychological and social background factors II Acta Paediatr Scand 66 729 1977.—Psychological factors were considered by the examining physicians to play an important part in 5 2% of approximately 4000 initial visits to a paediatric clinic These cases are compared with a matched control group representing the cases where the physicians judged that psychological factors were not involved As compared with the controls then a considerably larger proportion of the patients with symptoms associated with psychological factors had a previous history of contacts with child psychiatrists or attendence at the paediatric clinic for problems of a psychological nature Their parents the mothers in particular had a higher frequency of registered sickness for mental nervous disorders and their families had more frequently been the subject of special social inquiries or assistance To a large extent the examining paediatrician was unaware of these background conditions With a better case history and proper follow up one could probably reduce the number of X ray examinations consultations and laboratory tests Further training and better contacts would be facilitated if members of a children s psychiatric team were stationed within the paediatric clinic

KEY WORDS Children out patient psychological social parent morbidity

The material was obtained from a total of 3997 patients who during the course of one year made initial visits to the paediatric clinic in Umeå The material was comprised of 198 cases in which the examining paediatrician judged that psychological factors played a part (the PY group) 300 cases where the importance of psychological factors was doubtful (the PD group) and a matched control group of 544 cases comprising a sample of the cases where psychological factors were judged to be of no importance (the PN group) [1] The questions considered in this paper are chiefly whether these groups differ with respect to the child s earlier contacts with the health service family conditions the parents morbidity and the presence of social problems The purpose of the study and its materials and methods have been described in more detail in the previous paper (15) which also contains data on the children s actual visit to the clinic

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The following abbreviations are used in the text tables and figures PF psychological factor PY psychological factor Yes (i e of importance at the current visit) PD psychological factor Doubtful (i e doubtful whether important) PN psychological factor No (i e not important)

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Six months before the current visit the school nurse again reported that she had the same discomfort and had fainted during physical training She was referred to the child psychiatry clinic which she visited repeatedly during the next three months She was regarded as being depressed and as not having recovered from her father s death four years earlier There was also trouble with a boyfriend who was abusing alcohol She returned in the same month to the paediatric clinic with the same complaints as be fore—back pain nausea from apples eggs etc One month before the actual visit she attended on account of pain and stiffness in all joints tiredness and an inability to go to school There was still trouble with the boyfriend who had made a suicidal demonstration when she considered breaking off their relationship

Actual visit Arrived as an emergency case having awoken with a dull pain in the epigastrium and blood stained vomit in school Admitted to the ward where she was lively and alert Stomach pains at times which improved with liquid and antacids Laboratory tests provided no evidence of intestinal bleeding Further X rays of the stomach duodenum and lumbar spine showed nothing pathological neither did cholecystography

Subsequent history Returned a month later at night as an emergency referred from a local physician with numbness and paralysis in the legs difficulty in breathing and pains in the chest Clinical examination revealed noth ing pathological She was able to walk afterwards A new contact was arranged with the child psychiatry clinic with which she subsequently kept in touch for a long time

Abdominal pains causing admission to a de partment of surgery (with or without appen dectomy) had been noted in 6 6% of the PY group and in 3 9% of the PN group (n s)

Composition of the family and economic conditions Broken homes due to divorce or death were considerably more common in the PY group (12 6%) than in the PN group (4 6%) ($p<0\ 001$)

Mothers who had been under 20 years old when the patient was born were more com mon in the PY than in the PN group 12 3 and 4 8% respectively ($p<0\ 001$) *This difference* was not only common in the mothers attend ing with young children but even amongst the mothers with children in the older age groups (4–9 years and 10–15 years) *The PF* groups did not differ substantially in the breakdown of all the parents ages at the birth of the child

The PF groups did not differ significantly by the family income occupation or socio economic group (using the H M S O *Classification of Occupations*) (7) The proportion of mothers who were housewives or students (estimated as those with no taxable income) was 31 1% for PY and 35 1% for PN (n s)

Table 2 *Occurrence of the family in social registers in the period 1 1 1966–30 6 1971*

Percentage of PF groups

	PF group		Differ ence
	PY $N=198$	PN $N=544$	PY PN
Child welfare cases	17 6	5 0	
Social welfare cases	18 [illegible]	10 7	
Temperance cases	4 5	[illegible] 2	n s
Entry in social registers in the period 1 1 1970–30 6 1971	[illegible]1 2	11 0	

Parents morbidity The number of parents who had been registered sick for mental nerv ous complaints or disorders at some time dur ing the child s lifetime was significantly higher in the PY than in the PN group This also ap plied to the fathers and mothers separately The differences were marked for the parents of children aged 4–12 years while the fre quencies were similar for those aged 13–15 years (Fig 1)

The mothers in the PY group had a some what larger total number of sick days than those in the PN group while no such differ ence was found for the fathers

Social registers The families in the PY group featured in the social register consider ably more frequently than those in the PN group (Table 2)

Correlations Calculations were made of correlations for all the variables reported with the total material (PY+PD+PN)

The families which had been broken up by divorce or death had an increased incidence of registered sickness for mental nervous dis orders among both the fathers (20 6 against 6 1% $p<0\ 01$) and the mothers (31 8 against 10 1% $p<0\ 001$) and these families were over represented in the social register Families with a record of mental nervous disorders fea tured more frequently in the social register

Table 1 *Previous attendance at and/or admission to child psychiatry and paediatric clinics in Umeå in conjunction with clear or probable psychogenic complaints*

Percentage of PF groups

	PF group		Difference
	PY $N=198$	PN $N=544$	PY PN
Paediatric clinic	28 3	10 5	***
Child psychiatric clinic	16 2	3 1	*

METHOD

A search was made of the hospital in and out patient records on all the children attending at the departments of paediatrics and child psychiatry at Umeå hospital A count was made of earlier paediatric contacts and the case reports were classified by the author into three groups with regard to the importance of psychological factors—Yes Doubtful and No—using the wide definition of psychological factors described in the earlier article The surgical archives were searched and a record was made of the children who had been previously admitted for observation on account of abdominal pains

Data on the composition of the families the parents income occupations and registered sickness were obtained from official records A search was made for data on social investigations or measures over and above the community s normal service

RESULTS

Previous contacts with the health service

The PF groups did not differ statistically in the number of previous initial visits or emergency visits There were however many patients who had made several visits with different symptoms and problems

At previous initial visits or admissions many of the children particularly in the PY group presented problems which were judged to involve psychological factors (Table 1) This was particularly common among the older children

Even though they had a current contact with a child psychiatrist some children came to the paediatric clinic with emergency symptoms that had to be regarded as part of the psychiatric problem This is illustrated by the following case

Girl 15 years PD 313

Earlier history Seen as an emergency case when 14 years old There was a six months history of pains and numbness alternating between the arms and legs For about one week there had been a dull feeling of a lump and pains in the epigastrium She also presented a paresthesia in the right upper arm that did not correspond to the innervation fields *X ray examinations* of the stomach and duodenum were normal

A straightened lumbar lordosis had been found at an earlier X ray examination and she was referred to a physiotherapist for back trouble At a follow up many of the same complaints were noted and as a neurological disorder could not be ruled out she was admitted to the ward Neither *EEG ophthalmologic examination or electrophoresis of CSF* gave any indication of a neurological disorder She was referred to the child psychiatry clinic where it was found that her complaints had disappeared and there seemed to be no need for treatment Some weeks later the school nurse reported that the patient still had numbness headache and stabbing pains in the region of the heart besides subjective discomfort after the lumbar puncture She attended the paediatric clinic and was referred to an orthopedic surgeon who found poor posture and referred her once more to a physiotherapist

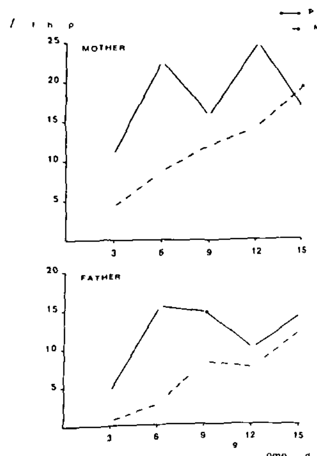


Fig 1 Parents sho had reported sick for mental nervous complaints or disorders during the child s lifetime Percentage of PY and PN groups at different ages of the child in complete years at actual visit

Six months before the current visit the school nurse again reported that she had the same discomfort and had fainted during physical training She was referred to the child psychiatry clinic which she visited repeatedly during the next three months She was regarded as being depressed and as not having recovered from her father's death four years earlier There was also trouble with a boyfriend who was abusing alcohol She returned in the same month to the paediatric clinic with the same complaints as be fore—back pain nausea from apples eggs etc One month before the actual visit she attended on account of pain and stiffness in all joints tiredness and an inability to go to school There was still trouble with the boyfriend who had made a suicidal demonstration when she considered breaking off their relationship

Actual visit Arrived as an emergency case having awoken with a dull pain in the epigastrium and blood stained vomit in school Admitted to the ward where she was lively and alert Stomach pains at times which improved with liquid and antacids Laboratory tests provided no evidence of intestinal bleeding Further Y *rays* of the stomach duodenum and lumbar spine showed nothing pathological neither did cholecystography

Subsequent history Returned a month later at night as an emergency referred from a local physician with numbness and paralysis in the legs difficulty in breathing and pains in the chest Clinical examination revealed noth ing pathological She was able to walk afterwards A new contact was arranged with the child psychiatry clinic with which she subsequently kept in touch for a long time

Abdominal pains causing admission to a de partment of surgery (with or without appen dectomy) had been noted in 6 6% of the PY group and in 3 9% of the PN group (n s)

Composition of the family and economic conditions Broken homes due to divorce or death were considerably more common in the PY group (12 6%) than in the PN group (4 6%) ($p<0\ 001$)

Mothers who had been under 20 years old when the patient was born were more com mon in the PY than in the PN group 12 3 and 4 8% respectively ($p<0\ 001$) This difference was not only common in the mothers attend ing with young children but even amongst the mothers with children in the older age groups (4–9 years and 10–15 years) The PF groups did not differ substantially in the breakdown of all the parents ages at the birth of the child

The PF groups did not differ significantly by the family income occupation or socio-economic group (using the H M S O *Classi*

Table 2 *Occurrence of the family in social registers in the period 1 1 1966–30 6 1971*

Percentage of PF groups

	PF group		Differ ence
	PY $N=198$	PN $N=544$	PY PN
Child welfare cases	12 6	5 0	
Social welfare cases	18 2	10 7	*
Temperance cases	4 5	2 ?	n s
Entry in social registers in the period 1 1 1970–30 6 1971	?1 2	11 0	

fication of Occupations) (7) The proportion of mothers who were housewives or students (estimated as those with no taxable income) was 31 1% for PY and 35 1% for PN (n s)

Parents morbidity The number of parents who had been registered sick for mental nerv ous complaints or disorders at some time dur ing the child s lifetime was significantly higher in the PY than in the PN group This also ap plied to the fathers and mothers separately The differences were marked for the parents of children aged 4–12 years while the fre quencies were similar for those aged 13–15 years (Fig 1)

The mothers in the PY group had a some what larger total number of sick days than those in the PN group while no such differ ence was found for the fathers

Social registers The families in the PY group featured in the social register consider ably more frequently than those in the PN group (Table 2)

Correlations Calculations were made of correlations for all the variables reported with the total material (PY+PD+PN)

The families which had been broken up by divorce or death had an increased incidence of registered sickness for mental nervous dis orders among both the fathers (20 6 against 6 1% $p<0\ 01$) and the mothers (31 8 against 10 1% $p<0\ 001$) and these families were over represented in the social register Families with a record of mental nervous disorders fea tured more frequently in the social register

(28 1 against 14 0% for the fathers, $p<0\ 01$ 30 3 against 15 3% for the mothers $p<0\ 001$) The fathers with a record of mental nervous disorders were overrepresented in the temperance register (18 8 against 2 1% $p<0\ 001$) and the mothers were overrepresented in the social assistance register (23 5 against 11 9%, $p<0\ 001$)

DISCUSSION

The material collected for this study covers practically all the contacts patients have had with paediatricians or child psychiatrists in the area served by the hospital

One can assume that the physician made a note of psychogenic complaints in the paediatric records if he considered them important but there were no doubt problems which escaped detection due to lack of doctor s time the presence of more prominent complaints or lack of contact with the patient This is in accordance with other reports (cf 8 11 17 25) The distribution by marital status parents age income occupation and the proportion of mothers who were housewives agrees with the contemporary study of families with children that was undertaken in Umeå (23)

Several studies have shown that children from broken homes run a greater risk of psychiatric problems and social maladjustment (3 12 22 28) and the relationship between parental deprivation in childhood and psychic disorders in adolescence and adulthood has been pointed out frequently (3 5 26 27) Very young mothers may constitute a risk which is probably due to the fact that their children are mostly unplanned (18) which in turn obstructs educational and occupational plans and impose a cohabitation for which the parents have not planned or are not sufficiently mature If allowance is made for the distribution of the parents by age and sickness benefit class the estimated mean number of sick days per year in the PN group did not differ appreciably from the frequency of registered sickness in the county of Vasterbotten in 1970 (20)

Many investigations have demonstrated a relationship between mental disorders in the parent and behavioural disturbances in their children (1 2 19 21) this also applies to lengthy somatic illness (2 21) Studies starting from children and adolescents with manifest disturbances and maladjustment have likewise shown an increased incidence of mental disorders in the parents (4 10 16 28)

Several investigations have indicated that paediatricians are often consulted for symptoms that may be a reaction to adverse social conditions (10 19) The registered sickness of the parents as a whole and for mental nervous disorders in particular displayed a strong positive correlation with entries in the social register This is in keeping with earlier investigations (13 24)

General discussion and recommendations

The attendance figures and the size of the population served by the hospital suggest that 15–20% of all children aged 0–15 years made one or several initial visits to the paediatric clinic There is thus a large need for help which parents and various bodies interpret as medical It is questionable whether the community s resources for health services and social welfare are correctly structured to meet the real needs of many of these families Studies have pointed out that the somatically inclined paediatrician may neglect to obtain important information about the patient s behaviour and situation information that may be crucial for an understanding of the illness or symptoms (6 17 25) The training of medical students in these matters is poor (8) and during their training they loose a good deal of their original ability to establish contact with parents (11) apparently because the training is excessively concerned with technical aspects

There is always some likelihood that the presenting symptoms have a somatic background and large resources are often invested—in the form of laboratory tests X ray examinations

nd consultations—in ruling out an organic ause These efforts cannot be dismissed in eneral as unnecessary but there are cases as llustrated in the present study which show hat many investigations could be cut down ubstantially if a more thorough history was btained and further personal contact was provided at a second visit Recommendations for better handling of these patients have re cently been suggested by Green (9)

Another advantage would be access to child guidance specialists (psychologist social worker and if possible child psychiatrist) with in the paediatric out patient clinic s own locali ties The patient could then be referred on a simple door to-door basis and the parents would not have to admit that they had been to see[6] psychiatrist In time such an integra tion would eliminate the prejudice against child psychiatry and the continuous contact between paediatric and psychiatric specialists would provide further training on a practical basis as well as further mutual understanding

Although these measures may appear time consuming they would certainly save the health service from many unnecessary new visits (often out of hours when costs are higher) and large investigations Hopefully one could also prevent certain cases from develop ing into serious psychiatric and social prob lems

Efforts should be made to benefit from a situation where the parents experience a need for help for which they attend with the child

ACKNOWLEDGEMENTS

Financial support to this study was obtained from the Bank of Sweden Tercentenary Fund (project 7 '69) and University of Umeå (Ungraduated Researchers Fund)

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Submitted Sept 27 1976
Accepted March 29 1977

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Acta Paediatr Scand 66 735–739 1977

FETAL RISKS DUE TO WARFARIN THERAPY DURING PREGNANCY

KARI O RAIVIO ENSIO IKONEN and SEPPO SAARIKOSKI

From the Children s and Women s Hospital University of Helsinki Helsinki Finland

ABSTRACT Raivio K O Ikonen E and Saarikoski S (Children s and Women s Hospital University of Helsinki Helsinki Finland) Fetal risks due to warfarin therapy during pregnancy Acta Paediatr Scand 66 735 1977 —Two mothers with heart valve prosthesis were treated with warfarin during pregnancy In the first case a caesarean section was done one week after replacement of warfarin with heparin The baby died of cerebral and pulmonary hemorrhage The second mother had a male infant by caesarean section The baby showed warfarin induced embryopathy with nasal hypoplasia and stippled epiphyses (chondrodysplasia punctata) Nasal hypoplasia with or without stippled epiphyses has now been reported in 11 infants born to mothers treated with warfarin during the first trimester and a causal association is probable In view of the risks to both mother and fetus in women with prosthetic cardiac valves it is recommended that therapeutic abortion be advised as the first alternative

KEY WORDS Warfarin embryopathy chondrodysplasia punctata

There is no doubt that pregnancy in a patient with a cardiac valve prosthesis should be discouraged because there are significant risks to both mother and fetus (4 8) When management of the pregnancy without anticoagulation has been attempted maternal complications (cardiac failure and embolization) have been unacceptably frequent and severe (4 17) On the other hand oral anticoagulant therapy with warfarin is definitely associated with an increased rate of abortion and perinatal mortality (4 17 18)

In recent years warfarin has been suspected of having a teratogenic effect (19) Nasal hypoplasia with or without radiologically demonstrable epiphyseal stippling (chondrodysplasia punctata) has been described in at least 10 infants whose mothers had been treated with warfarin during the first trimester of pregnancy

The purpose of this report is to contribute further information relating warfarin administration with chondrodysplasia punctata and to illustrate other risks to mother and fetus in a woman with a cardiac valve prosthesis

CASE REPORTS

Case 1

This case has been briefly described previously (7) The mitral valve of the mother was replaced with a prosthesis 4 mon hs before conception She was maintained on digitalis and warfarin Against medical advice she became pregnant and concealed the fact until the 30th week At that time she was admitted to the hospital because of the cardiac problem and toxaemia of pregnancy (blood pressure 170/120 oedema albuminuria 0 5%) In both respects her condition stabilized thrombotest levels were 15–39 At the beginning of the 35th week oral warfarin was replaced by subcutaneous heparin which was to be continued until term However one week later severe exacerbation of the toxaemia (blood pressure 230/110 low urinary estriol elevated BUN) necessitated termination of the pregnancy by caesarean section

The mother had no postoperative cardiac problems

The male infant weighed 2 160 g at birth and the Apgar score was 7 and 10 at one and seven minutes respectively He had a broad and flat nasal bridge but no other abnormalities Vitamin K (1 mg) was administered

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Fig 1 The infant of Case 2 showing nasal hypoplasia and relative shortness of the proximal extremities

Thrombotest was subsequently found to be 19% but clotting and bleeding times were normal The baby did well for 4 hours but then stopped breathing Mechanical ventilation was commenced and had to be continued until death at the age of 3 days 18 hours Autopsy revealed cerebral and pulmonary haemorrhage and septicaemia due to Staphylococcus aureus

Examination of the X rays showed that there was no epiphyseal stippling in the vertebral column or upper extremities but the lower extremities were not studied

Case 2

The mother was 38 years old and had rheumatic heart disease Her calcified mitral valve had been replaced with a prosthesis at the age of 34 years after which she was given continuous warfarin therapy

At the start of the pregnancy three weeks after the last menstrual period the patient had an acute myocardial infarction Recovery was uneventful but because of the possibility of coronary embolism exceptionally low Thrombotest levels (8–13%) were subsequently maintained with warfarin Other medication included digoxin furosemide spironolactone and supplementary potassium

The patient was first seen at the obstetric clinic and the pregnancy was confirmed during the 15th week of gestation She refused therapeutic abortion

Except for a slight degree of polyhydramnios the pregnancy proceeded normally The mother was hospitalized at the 35th week warfarin was discontinued and intravenous heparin started During the 37th week radiographs of the fetus showed marked epiphyseal stippling in the vertebrae proximal and distal humeri and ankle regions On the basis of these findings a prenatal diagnosis of chondrodysplasia punctata was established Uterine contractions started spontaneously at the end of the 37th week but because of signs of asphyxia a caesarean section was performed

After the operation the mother developed septicaemia due to Staphylococcus aureus and was in a critical condition for 4 weeks Complications included left sided hemiplegia presumably due to septic emboli She survived with markedly impaired function in the left arm and leg and some speech impairment

The male infant had a birth weight of 2 150 g a length of 44 cm and a head circumference of 36 cm The Apgar score was 5 and 8 at one and ten minutes respectively The peculiar facial features were immediately noted (Fig 1) The nose was flattened and small the nasal bridge was broad the nares were small but a thin catheter could be passed through both choanae A bilateral simian crease was present but there was no brachydactyly and other malformations were not detected Ophthalmological examination was normal The baby had respiratory difficulties and severe CO_2 retention during the first hours of life but these were relieved by an oropharyngeal airway Vitamin K and fresh frozen plasma were administered during the first day of life and there were no signs of bleeding

Except for the skeletal changes several chest X rays and plain films of the abdomen were all normal The characteristic stippling was seen in and around the vertebral bodies especially the sacrum (Fig 2) in the tarsal and carpal bones and inferior parts of the pelvis and smaller clusters were present in the scapulae proximal and distal humeri and around the femoral heads There were no metaphyseal changes but the humeri and femora were short relative to the radii and tibiae Bone structure and the degree of calcification appeared normal The skull was radiologically normal

Laboratory studies on the first day of life revealed normal serum calcium phosphate and alkaline phosphatase values Urinary amino acid excretion was elevated but the pattern was nonspecific No phosphoethanolamine was detected Urinary hydroxyproline excretion wa 0 096 mmol/day which is below the normal range

After the initial respiratory difficulties and feeding problems the baby did relatively well At the age of 3 weeks tachypnoea and breathing problems reappeared and the baby died at the age of 24 days In addition to the skeletal lesions autopsy revealed diffuse bilateral bronchopneumonia

DISCUSSION

It seems unavoidable that patients requiring anticoagulant treatment will continue to be encountered in antenatal clinics because neither cardiac valve prosthesis (4 17) nor deep ve

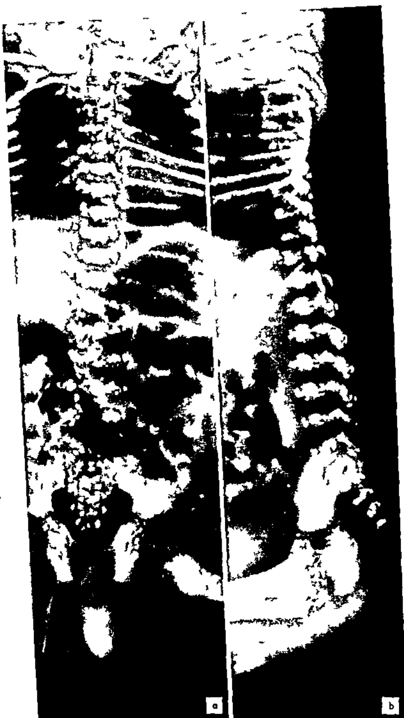


Fig. 2. Skeletal X ray findings in the infant of Case ?



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Submitted Jan 3 *1977*
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Table 1 *Incidence of major clinical features in 11 cases with presumed warfarin induced embryopathy (references 1 9 12–15 17 plus the present Case 2)*

Previous abortions during warfarin therapy	6/11
Pregnancy complications	5/10
Low birth weight (–2 S D or less)	3/11
Mental retardation	3/6
Nasal hypoplasia	11/11
Respiratory difficulties	7/8
Eye abnormalities	4/9
Stippled epiphyses	8/9
Brachydactyly	2/6
Other gross malformations	1/11

nous thrombosis with or without pulmonary embolism (6 18) can be safely managed otherwise

The recommendation to use heparin instead of warfarin during the first trimester and the last weeks of pregnancy appears to be well founded (4 6 8 11) However, in practice this is difficult to follow Women on continuous oral anticoagulation may not recognize or report their pregnancy until late in the first trimester or even later In later pregnancy premature delivery may upset the plans for altering drug treatment as happened in our Case 1

Warfarin has a number of potential harmful effects on the fetus In accord with animal studies (5 10) mothers treated with warfarin have a high incidence of abortions and still births Out of 55 such pregnancies reviewed by Harrison & Roschke (4) 16 (29%) terminated in abortion or stillbirth and there were 4 neonatal deaths Out of the 11 warfarin treated mothers delivering a child with the chondrodysplasia punctata syndrome (Table 1) 6 had a history of previous abortions Our Case 2 had 4 spontaneous abortions during warfarin therapy whereas prior to this treatment she had two normal pregnancies and one therapeutic abortion

The syndrome of nasal hypoplasia with or without stippled epiphyses chondrodysplasia punctata has now been recognized in 11 infants born to warfarin treated mothers (Table 1) Since the nasal deformity is obvious at birth and relatively uncommon it should constitute an indication for careful search for chondrodysplasia punctata

On a clinical radiological and genetic basis Spranger et al (16) have subdivided chondrodysplasia punctata into two main types the Conradi–Hunerman type and the rhizomelic type Affected infants of warfarin treated mothers can be regarded as phenocopies of the former usually dominantly inherited type which clearly is not a homogeneous entity

Our experience with the two cases suggests that the effect of warfarin on the fetus may be dose dependent The thrombotest level in Case 2 was maintained significantly lower than that in Case 1 and her infant exhibited the complete syndrome However as pointed out by Hall (3) the fetal effects of warfarin are difficult to relate to its classical anticoagulant action since clotting factors are not present in first trimester embryos (2)

After the reconstruction of a damaged heart valve the condition of a severely and chronically ill patient improves and she feels optimistic about reproduction However in view of the grave basic heart lesion prospects for long term normal motherhood must be guarded When the fetal risks of abortion malformations and perinatal death are also taken into account we feel that at present a thorough explanation of the problems and unequivocal advice against conception must be given to any woman of childbearing age who is about to undergo valve replacement

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Submitted Jan 3 1977
Accepted March 11 1977

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Acta Pædiatr Scand 66 741–744 1977

THE GROWTH OF THE KIDNEYS IN CHILDREN WITH VESICOURETERIC REFLUX

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ABSTRACT Kaas Ibsen K Uldall P and Frøkjær O (University Clinic of Paediatrics Children s Hospital Fuglebakken Copenhagen Denmark) The growth of the kidneys in children with vesicoureteric reflux Acta Paediatr Scand 66 741 1977.—In a retrospective study 69 kidneys with VUR were divided into 2 groups One group (A) where VUR stopped within 1 year after operation or conservative treatment and a second group (B) where VUR continued for more than 1 year Group A had somewhat more severe grades of reflux than group B The number of infections were practically the same in the two groups The length of the kidneys was measured at the time of diagnosis and compared with the length at the most recent urography after VUR stopped (group A) on average 2 years and 3 months later and with the most recent urography while VUR was still present (group B) on average 1 year and 9 months later It was found that 85% in group A had increased in absolute length while the figure was 60% in group B If the relative growth is calculated (the kidneys length in relationship to L_1-L_3 distance) 60% in group B had decreased while only 30% in group A had decreased

KEY WORDS Children kidney growth vesicoureteric reflux urinary tract infection

The indications for operation for vesico ureteric reflux (VUR) are still under discus sion The purpose of this study is to contribute to the discussion by describing the results found by following the kidneys in one group of patients with persistent VUR for more than one year and in another comparable group where VUR stopped within one year

MATERIAL AND METHODS

From January 1964–December 1974 a total of 76 children were diagnosed as having VUR (173 ureters with reflux) at the Children s Hospital Fuglebakken In the present study a child whose operation for VUR failed a child with stenosis of urethra and children with an insufficient number of urography examinations or where it was impos sible to measure the length of the kidney (30 kidneys) were excluded Further the material was restricted to kidneys of average size (± standard deviations) at the time of diagnosis since abnormal kidneys may be anticipated to behave differently from those of normal size At the time of diagnosis 20 kidneys were not within ±2 standard deviations Of the kidneys being above 2 standard devia tions four had hydronephrosis two duplication of the pelvis three had compensatory growth while in four kidneys no obvious explanation could be found Of the kidneys which were too small all were described as having severe chronic pyelonephritis with one exception Thus the material used in this investigation comprises 69 kidneys with VUR

As the study is retrospective the number of urographies on the patients varied When comparing the lengths of the kidneys the most recent urographies were always used The diagnosis of VUR was made by cystourethrography and all examinations except four were assessed by the same radiologist Each individual patient s urographies were analysed by measuring the length of the kidney and the distance from the upper edge of the first lumbar ver tebra to the lower edge of the third lumbar vertebra

Eklof & Ringertz (?) have drawn up a nomogram in which given the length of the kidney and the distance between L_1-L_3 it is possible to calculate how much a given length of the kidney deviates in standard deviations from an average value The size of the kidneys and the distance from L_1-L_3 were introduced in this nomogram This procedure is found to be approximate as the normal values for the length of the kidneys vary greatly

Table 1 *The age and sex distribution of the material*

Age in years	♀	♂
0–1	10	9
2–3	3	3
4–5	12	2
6–7	5	0
8–9	2	1
10–11	1	1
Total	33	16

RESULTS

The sex and age distribution appears in Table 1

The kidneys were divided into two comparable groups In group A VUR stopped within one year Eleven of these 39 kidneys were treated conservatively with long term antibiotic treatment while the rest were operated upon on average three months after the diagnosis Group B consists of 30 kidneys where VUR continued for more than one year Twelve of these kidneys were subsequently operated upon while the rest were treated with long term antibiotic treatment The duration of reflux from the time of diagnosis until the latest urography used for measuring while reflux was still shown on micturition cystourethrography was 1 year and 9 months on average

In order to compare the two groups some factors which could be expected to influence the growth of the kidneys were registered The grading of reflux is modified after the system described by Rolleston et al (6) This modification (8) was made as more than half of the material would otherwise have been placed in the middle group (moderate reflux) which we found unclearly defined The modified graduation appears in Table 2 It appears that there were more kidneys with severe grades of VUR in group A than group B

The number of infections (more than 10^5 bacteria per ml) during the period from diagnosis until VUR stopped was on an average 2 in group A In group B 2–3 infections were found from the time of diagnosis until the last urography used Further no significant difference was found between the two groups concerning age and sex

Table 3 summarises the growth of the kidneys in the two groups By relative growth is understood the kidney s length is increased in relationship to L_1-L_3 distance By absolute growth is understood an increase in mm On account of uncertain measurement changes of less than 2 mm are described as unaltered size

In group A the urography at the time of diagnosis is compared with the most recent *urography after VUR stopped* (a period of 2 years and 3 months on an average) In group B

Table 2 *The modified graduation and the distribution of the material*

Degree of VUR	(A) Kidneys with VUR for less than one year	(B) Kidneys with persistent VUR for more than one year
I Incomplete filling of the uretero-pelvine system	5	4
II Complete filling without dilatation of the uretero-pelvine system	4	9
III Complete filling and dilatation of the uretero pelvine system	19	13
IV The same as III but with distension of the calyces	11	4
Total	39	30

Table 3 *The growth of the kidneys*

	Group A Kidneys with VUR for less than one year		Group B Kidneys with persistent VUR for more than one year	
	Absolute	Relative	Absolute	Relative
Increased	33 85%	13 33%	20 66 7%	4 13%
Unchanged	? 5%	14 36%	5 16 6%	8 27%
Decreased	4 10%	12 31%	5 16 6%	18 60%
Total	39 100%	39 100%	30 100%	30 100%

the first urography is compared with the most recent urography while VUR is still present (on an average 1 year 9 months later)

As can be seen in Table 3 absolute growth occurred in most of the kidneys in both groups Eighty five percent in group A had increased length while the figure was 60% in group B If the relative growth is calculated 60% in group B had decreased while only 30% in group A had decreased

DISCUSSION

Change in the length of the kidney (maximal length measured from pole to pole on a urography X ray picture) is usually a satisfactory measurement for the complete growth of the kidney This occurs normally at a more or less constant rate from 4–15 years of age The kidneys in each individual have normally the same rate of growth and the difference in length in the two kidneys rarely exceeds 5 mm (3) From his observations on the length of the kidney Hodson (3) made a curve that shows directly the length of the kidney in children 0–16 years of age No significant difference in the size of the kidneys was found between the two sexes

From Hodson s curve and his own measurements Currarino (1) found a constant relationship between the length of the kidney and the distance from the upper edge of the first lumbar vertebra to the lower edge of fourth lumbar vertebra (L_1-L_4 distance) However this does not apply during the first 1–1½ years of life

Eklof & Ringertz (2) have made a very practical nomogram also using the lumbar vertebra as reference to the kidney length This system has been used in this investigation

McLachlan et al (4) found that the length of the kidney in children with VUR is very close to the average length of the kidney stated by Hodson In our material about 80% was of average size

In one of the very few prospective studies Scott & Stansfeld (7) examined a total of 58 patients with VUR who were divided into a group submitted to operation and a control group It was found that the increase in size of the kidney of the operated cases where the reflux was stopped by operation was nearly four times as large as the increase in the control group with persistent reflux Our trial is based on a somewhat different material We have for instance included kidneys where VUR stopped without operation but our results seem however also to support the idea that kidneys with VUR seem to grow less constantly This is especially marked when the relative growth is calculated Our results are reinforced by the fact that in group A more kidneys than in group B belong to the more severe grades of reflux Furthermore the number of infections in the two groups was practically identical

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Acta Pædiatr Scand 66 745–751 1977

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INDICATION OF PRIMARY IMMUNE DEFICIENCY IN FANCONI S ANEMIA

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ABSTRACT Pedersen F K Hertz H Lundsteen C Platz P and Thomsen M (University Clinic of Paediatrics Department G Section of Teratology Department of Obstetrics and Gynecology and Department of Paediatrics and Tissue Typing Laboratory Rigshospitalet Copenhagen Denmark) Indication of primary immune deficiency in Fanconi s anemia Acta Paediatr Scand 66 745 1977 —A girl with various congenital malformations developed pancytopenia and hypoplastic bone marrow at the age of 6 years A chromosome study of lymphocytes showed numerous breaks gaps and rearrangements allowing the diagnosis of Fanconi s anemia Treatment with corticosteroids and splenectomy did not result in hematologi remission Repeated immunologic studies showed increasingly deficient T cell function as judged by lymphocyte transformation studies and skin test reactivity whereas T cell number T/B cell ratio immunoglobulins complement factors and neutrophil function were normal A severe Pneumocystis carinii pneumonitis developed but was successfully treated with pentamidine sulfametoxazole with trimetoprim and transfer factor Improvement of T cell function followed transfer factor therapy Combined therapy with corticosteroids and androgens caused partial remission of the hematologic abnormalities The probability of a primary immune deficiency in the patient is discussed

KEY WORDS Fanconi s anemia chromosome aberrations immune deficiency Pneumocystis carinii pneumonitis transfer factor therapy

The designation Faconi s anemia has been used for cases of multiple congenital malformations and bone marrow failure since Fanconi s original report of 3 patients from one family in 1927 (7) Studies from the last 10 years have shown that these patients in addition often have chromosome aberrations such as breaks gaps and rearrangements (5) as well as an increased incidence of malignancies (6)

More than 160 cases familial or sporadic have been published (4) Though frequent infections are part of the symptomatology these have generally been attributed to the neutropenia and reports that include full immunologic work up in patients with typical Fanconi s anemia are not known to us

This report presents a case of typical Fanconi s anemia associated with in vitro T lymphocyte deficiency which clinically manifested itself in a *Pneumocystis carinii* pneumonitis

CASE REPORT

Patient T E a 6-year-old girl was admitted in August 1974 due to recurrent infections of the skin and bleeding tendency of 5 months duration

Three grandparents died of cancer in the stomach rectum and breast respectively A sister of the father died neonatally with severe malformations of unknown kind

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Previous reports (5 6) have shown that pro nounced VUR must be present to affect the growth of the kidneys severely and to cause progressive renal damage

Even though this undoubtedly is true from our studies the growth of kidneys with persist ent VUR seems to be somewhat slower than of those where VUR stops regardless that the latter group had more severe grades of reflux However, the majority of kidneys with persist ent VUR seems to grow well and it would therefore seem natural to recommend measur ing the kidney growth for instance with a no mogram of Eklof & Ringertz (2) before deci sion is taken whether or not operation should be undertaken

ACKNOWLEDGEMENT

We thank Dr M Eiken for assistance in assessing difficult X rays and Dr K E Petersen for assistance with the manuscript

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Submitted Oct 5 1976
Accepted May 27 1977

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Acta Pædiatr Scand 66 745–751 1977

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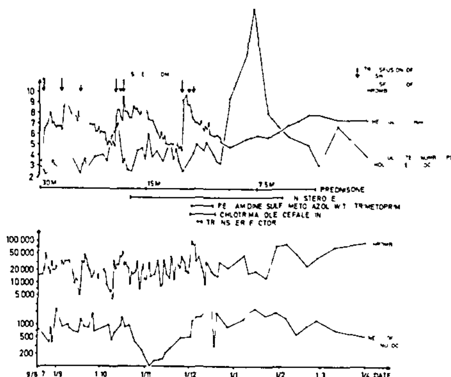


Fig 1 Hematologic paramters in relation to treatment during the period of investigation

and the father himself had pulmonary tuberculosis a decade ago but otherwise cases of congenital malformations severe infections or hematologic diseases were not known to have occurred in the family Two siblings 8 and 2 years old are in good health do not have malformations and both have normal chromosome studies

The pregnancy was normal delivery uncomplicated at the 36th gestational week and birth weight was 2400 g A supernumerary first left finger was removed in the neonatal period and 3 months old the patient underwent surgery for a persistent ductus arteriosus and an aberrant branch of the left pulmonary artery A hearing loss due to atresia of the left external auditory canal and abnormalities of the bony structures of the right middle ear was present Physical development was characterized by height weight and head circumference just below the 10th percentile for age

The patient had uncomplicated chickenpox and measles between the age of 3 and 4 years but never had mumps and in general did not have any increased frequency of infections before the present episode She had never received live vaccines The last 5 months before admission she developed recurrent infections of the skin caused by *Staphylococcus aureus* and *Candida albicans* in addition to bleeding tendency

Physical examination on admission in addition to the above mentioned congenital malformations showed a pale girl with numerous petecchiae and ecchymoses in the skin where also 4 cafe au lait coloured spots were noted There was an oral moniliasis but no other evidence of actual infection Mental development was normal

The following studies were carried out

Hematologic studies On admission hemoglobin concentration was 3 3 mmol/l reticulocytes 0 8% of erythrocytes thrombocytes 15×10^9/l and leucocytes $4\ 8\times10^9$/l A differential count of leucocytes showed 16% neutrophil granulocytes 82% lymphocytes and 2% monocytes Subsequent figures for hemoglobin reticulocytes thrombocytes and neutrophil granulocytes are shown in Fig 1 Erythrocyte mean cell volume was $115\mu^3$ and mean cell hemoglobin concentration 20 mmol/l Serum folate was 24 nmol/l serum B_{12} 888 pmol/l and fetal hemoglobin 21% of total hemoglobin Serum bilirubin and haptoglobin were normal Coombs test was negative and antibodies against leucocytes and thrombocytes were not found

The bone marrow was hypoplastic Erythropoesis initially was of normal intensity later sparse but still normoblastic Myelopoesis was sparse but contained normally structured cells from all stages of development Megakaryocytes were not seen Bone marrow culture did not reveal any colony forming cells and colony stimulating factor in serum could not be demonstrated

Cytogenetic studies Metaphase chromosomes were produced in a standard manner from peripheral blood and stained with orcein or trypsin Leishman One hundred orcein stained and one hundred trypsin Leishman stained mitoses were analysed A total of 73 mitoses (36%) showed aberrations There were 20 mitoses with chromatid gaps 31 with chromatid breaks 5 with chromatid rearrangements 4 with acentric fragments and 13 mitoses showed aberrant distribution of chromosome types within the cell

No dicentrics tricentrics rings endoreduplications or polyploids were seen Using the Latt technique the frequency of sister chromatid exchanges was not found to be increased (12) Fig 2 shows characteristic examples of the aberrations found

Immunologic studies The following in vitro tests were



Fig 2 Characteristic examples of chromosome aberrations found chromatid breaks and gaps (*a*) chromatid rearrangements (*b*) acentric fragment (*c*) and unidentified structure (*d*)

performed repeatedly (*a*) determination of T and B cells in peripheral blood by rosette formation with sheep erythrocytes (E rosettes T cells normal range 5[illegible]–80%) and with complement coated erythrocytes (EAC rosettes B cells normal range 8–[illegible]9%) and by direct membrane immunofluorescence for immunoglobulin bearing cells (Ig+ cells B cells normal range 8–[illegible]8%) (*b*) lymphocyte transformation with the mitogens phytohemagglutinin A (PHA Difco) and poke weed mitogen (PWM Gibco) and the antigens tuberculin (PPD State Serum Institute Copenhagen) Candida albicans (water soluble extract) Staphylococcus aureus and Escherichia coli (both heat

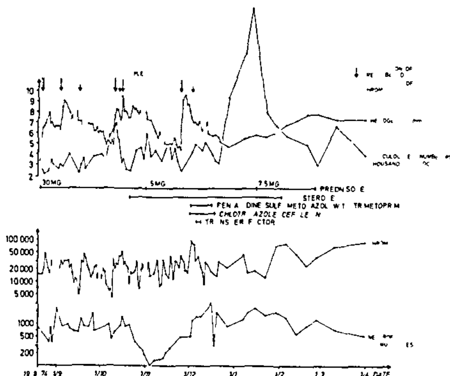


Fig 1 Hematologic paramters in relation to treatment during the period of investigation

and the father himself had pulmonary tuberculosis a decade ago but otherwise cases of congenital malformations severe infections or hematologic diseases were not known to have occurred in the family Two siblings 8 and 2 years old are in good health do not have malformations and both have normal chromosome studies

The pregnancy was normal delivery uncomplicated at the 36th gestational week and birth weight was 2400 g A supernumerary first left finger was removed in the neonatal period and 3 months old the patient underwent surgery for a persistent ductus arteriosus and an aberrant branch of the left pulmonary artery A hearing loss due to atresia of the left external auditory canal and abnormalities of the bony structures of the right middle ear was present Physical development was characterized by height weight and head circumference just below the 10th percentile for age

The patient had uncomplicated chickenpox and measles between the age of 3 and 4 years but never had mumps and in general did not have any increased frequency of infections before the present episode She had never received live vaccines The last 5 months before admission she developed recurrent infections of the skin caused by *Staphylococcus aureus* and *Candida albicans* in addition to bleeding tendency

Physical examination on admission in addition to the above mentioned congenital malformations showed a pale girl with numerous petecchiae and ecchymoses in the skin where also 4 cafe au lait coloured spots were noted There was an oral moniliasis but no other evidence of actual infection Mental development was normal

The following studies were carried out

Hematologic studies On admission hemoglobin concentration was 3 3 mmol/l reticulocytes 0 8% of erythrocytes thrombocytes 15×10^9/l and leucocytes 4.8×10^9/l A differential count of leucocytes showed 16% neutrophil granulocytes 82% lymphocytes and 2% monocytes Subsequent figures for hemoglobin reticulocytes thrombocytes and neutrophil granulocytes are shown in Fig 1 Erythrocyte mean cell volume was $115\mu^3$ and mean cell hemoglobin concentration 20 mmol/l Serum folate was 24 nmol/l serum B_{12} 888 pmol/l and fetal hemoglobin 21% of total hemoglobin Serum bilirubin and haptoglobin were normal Coombs test was negative and antibodies against leucocytes and thrombocytes were not found

The bone marrow was hypoplastic Erythropoesis initially was of normal intensity later sparse but still normoblastic Myelopoesis was sparse but contained normally structured cells from all stages of development Megakaryocytes were not seen Bone marrow culture did not reveal any colony forming cells and colony stimulating factor in serum could not be demonstrated

Cytogenetic studies Metaphase chromosomes were produced in a standard manner from peripheral blood and stained with orcein or trypsin Leishman One hundred orcein stained and one hundred trypsin Leishman stained mitoses were analysed A total of 73 mitoses (36%) showed aberrations There were 20 mitoses with chromatid gaps 31 with chromatid breaks 5 with chromatid rearrangements 4 with acentric fragments and 13 mitoses showed aberrant distribution of chromosome types within the cell

No dicentrics tricentrics rings endoreduplications or polyploids were seen Using the Latt technique the frequency of sister chromatid exchanges was not found to be increased (12) Fig 2 shows characteristic examples of the aberrations found

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Fig. 2. Characteristic examples of chromosome aberrations found: chromatid breaks and gaps (*a*), chromatid rearrangements (*b*), acentric fragment (*c*) and unidentified structure (*d*).

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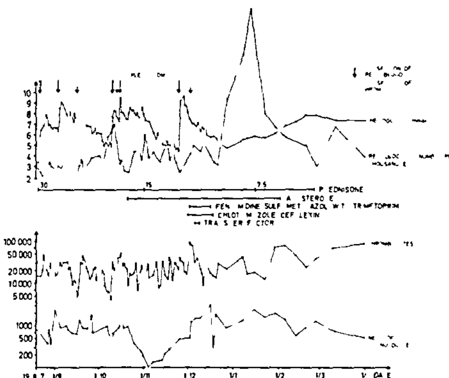


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combined therapy with prednisone and anasterone was started. A partial hematologic remission occurred after 9 weeks therapy as manifested by normalization of hemoglobin concentration and stabilisation of thrombocyte particle concentration at a somewhat higher level whereas neutrophil particle concentration remained relatively unchanged.

The patient had a persistent oral moniliasis that could be suppressed by local treatment with nystatin but recurred as soon as the drug was stopped. After 12-13 weeks in the hospital she developed a life threatening interstitial pneumonitis as manifested by fever around 39 degrees Celsius, dry cough, severe tachypnoe, cyanosis, normal lung stetoscopy but diffuse confluent infiltration in both lung fields on chest X ray. Therapy with sulfametoxazole with trimetoprim, cefalexin, clotrimazole and pentamidine aiming at bacterial as well as fungal and Pneumocystis carinii etiology was initiated. Cultures from tracheal aspirates however were and remained negative for bacteria and fungi and repeated serologic studies gave no evidence of viral infection. Microscopic examination of tracheal aspirate in Gomori's methenamine stain showed as seen in Fig. 4 accumulation of foam like material containing cysts of approximately 2 μ s size with a black coloured outer membrane and a dark staining central core allowing the diagnosis of Pneumocystis carinii infection. In an effort to improve T cell function transfer factor prepared from a pool from normal donors was given on 3 consecutive days in doses of 1, ?, and 3 ampouls respectively, each ampoul containing transfer factor corresponding to 0.3×10^9 leucocytes. On completion of transfer factor therapy, when sulfametoxazole with trimetoprim 50 mg/kg/24 h and pentamidine 4 mg/kg/24 h had been given for 6 days, temperature returned to normal. Respiratory distress disappeared a few days later and the two drugs could be discontinued after 2 weeks therapy. Though clinically without evidence of pulmonary infection the patient's bilateral pulmonary infiltrates on X ray persisted for approximately one year before complete resolution occurred.

Transfer factor therapy and cessation of antibiotic treatment was followed by improvement in T cell function as seen in Fig. 3 and described above.

DISCUSSION

Supernumerary thumb, congenital heart disease, middle and external ear malformations, growth retardation and cafe au lait spots of the skin all have been reported previously in patients with Fanconi's anemia (4). The chromosome findings described are not specific for this disease and have been seen also in patients with ataxia teleangiectasia, Bloom's syndrome, glutathion reductase deficiency, pernicious anemia and after irradiation (22). Our patient however showed no clinical signs of these conditions and the combination of congenital malformations, bone marrow failure and chromosome aberrations is definite evidence of Fanconi's anemia. A poor response to therapy with corticosteroids alone and splenectomy but occurrence of partial hematologic remission after 2–3 months of combined treatment with corticosteroids and androgens also is characteristic for this disease (18).

Infection with the protozoan *Pneumocystis carinii* typically gives rise to a severe interstitial pneumonitis. The organism is known to cause disease only in patients with impaired immune response and has been reported in premature and malnourished infants below 4 months, in children with congenital immune deficiencies and in patients with acquired immune deficiencies due to malignant disease or massive immunosuppressive therapy as for example after transplantation (23). Treatment with pentamidine isothionate reduces lethality to approximately 30% (13) and recent reports indicate that sulfamethoxazole with trimetoprim also is effective (15).

Pneumocystis carinii infection, recurrent oral moniliasis, skin reactivity and in vitro tests for T cell function point to a deficient cellular immunity in our patient. Whether this represents a primary immune deficiency or is a therapy induced secondary phenomenon is quite complicated to judge.

Corticosteroids may impair the response to skin tests and are also known to influence lymphocyte function. However they affect the PHA response of various subpopulations of lymphocytes differently thus increasing the response of bone marrow lymphocytes and medullary thymus cells but diminishing that of splenic lymphocytes (3) and the resulting effect on transformation of the total lymphocyte population of peripheral blood therefore may be only minor. Splenectomy is known to decrease the response of lymphocytes to transformation with as well mitogens as antigens in the immediate postoperative period but in a recent study the response was

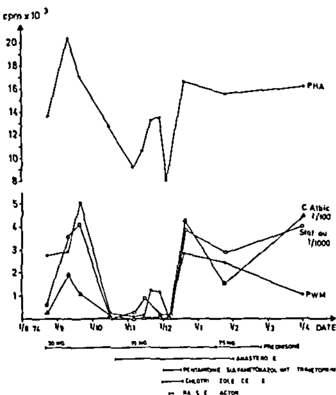


Fig 3 Results of lymphocyte transformation tests using phytohemagglutinin (PHA) and poke weed mitogen (PWM) as mitogens and Candida albicans and Staphylococcus aureus as antigens The lower normal value for stimulation with PHA is 14000 increment counts per minute (cpm) with PWM 3500 and with the antigens 1000 cpm All figures are median values of triplicates

killed organisms) All methods are described in detail elsewhere (20)

Initially normal percentages of E rosettes EAC rosettes and Ig+ cells were found E and EAC rosettes remained within normal range throughout the period of observation with a variation from 66–82% (1 54–6 22×10⁹/l E rosette forming lymphocytes) and 9–24% (0 39–1 63×10⁹/l EAC rosette forming lymphocytes) respectively Ig+ cells on two occasions November 20th and December 3rd however were very low 2% and 1% (0 07 and 0 02×10⁹/l) respectively whereas EAC rosettes on the same dates were 13% and 23% (0 44 and 0 43×10⁹/l)

Fig 3 shows the results obtained from the lymphocyte transformation tests with PHA PWM *Candida albicans* and *Staphylococcus aureus* The response to *Escherichia coli* followed that to *Staphylococcus aureus* and the response to PPD was negative It is seen that the results fall in three parts At the first 3 investigations in August and September normal to borderline transformation was found From the middle of October to the beginning of December that means before any clinical evidence of infection was present the response to mitogens as well as to antigens was severely depressed After treatment with transfer factor pentamidine and antibiotics as described below lymphocytes showed a normal transformation response to PHA and antigens but the response to PWM remained subnormal

The concentration of immunoglobulins in serum was normal IgG 14 5 g/l IgA 1 19 g/l IgM 0 64 g/l and IgE 100 μg/l Also the concentration of adenosine deaminase in erythrocytes was normal

Unstimulated and stimulated nitro blue tetrazolium test (NBT test) were normal and the same was true of granulocyte myeloperoxidase In vitro examination of neutrophil capability of uptake and intracellular killing of *Staphylococcus aureus* showed no signs of significant primary defect in neutrophil function (14)

Concentration of complement factors C_{1q} C_{1s} C_3 proactivator C_3 C_4 and C_5 and total hemolytic complement in serum all were normal (16 17)

Skin tests performed during the 3rd week after initiation of prednisone therapy with PPD streptokinase/streptodornase and Candida albicans antigen in dilution 1 1000 and 1 100 were negative whereas Candida albicans antigen in dilution 1 10 caused a positive reaction with a skin infiltration of 16 mm in diameter Sensitization with 0 05 ml 10% solution of 2 4-dinitrochlorobenzene in acetone applied to the forearm on a filter paper 1 cm in diameter for 12 hours was not followed by reaction on challenge with a similar 0 1% solution applied in the same manner 16 days later

Other studies Initially cultures from nose and throat trachea blood urine and stools were negative for pathogenic bacteria but *Candida albicans* was grown from the throat Virus could not be cultured from throat washings or stools by routine methods Antibody titers against Influenza virus A B and C Adeno virus Respiratory syncytial virus Herpes simplex virus Parotitis virus Mycoplasma and Ornithose agent did not give any evidence of recent or actual infection and hepatitis associated antigen was not found Morbilli virus complement fixation titer was positive in the dilution 1 32

Chest X ray skull X ray and an intravenous pyelogram were normal

As seen from Fig 1 prednisone alone in doses of 2 mg/kg/24 h and splenectomy did not affect the pancytopenia and on the basis of a diagnosis of Fanconi s anemia



Fig 4 Pneumocystis carinii cysts in tracheal aspirate Gomori s methenamine stain original magnification ×100

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found largely normalized 10 days postoperatively (2) In our case the first observed severely depressed lymphocyte transformation study (on October 14th) was made just before splenectomy and furthermore T cell function remained severely depressed quite far beyond the 10th postoperative day Antibiotic therapy that at least in case of sulfametoxazole with trimetoprim may decrease the response of lymphocytes to transformation with PHA (8) had not yet been started when pronounced cellular immune deficiency developed Viral infection which is known to influence cellular immunity in certain instances (24) was not present as judged by clinical examination, virus cultures and serologic studies In conclusion we therefore find that the evidence is in favor of a primary immune deficiency rather than a purely therapy induced immune suppression though the latter cannot be quite excluded The variable degree of depression of T cell function initially may be accounted for if it is assumed that the deficiency of cellular immunity in Fanconi s anemia like the hematologic changes develop gradually at a certain age This would also be in accordance with the fact that our patient had measles and chickenpox without complications in earlier life

On two occasions a discrepancy between EAC rosettes and Ig+ cells were observed Both methods for estimation of B lymphocytes correlate well in our hands in normal individuals but may give different results in patients with immune deficiencies Yata & Tsukimoto (25) in patients with Bruton s agammaglobulinemia found that these patients lack lymphocytes with surface immunoglobulins but have normal frequency of complement C_3 receptor bearing (and thus EAC rosette forming) lymphocytes and suggest that this receptor occurs at an early maturation stage of B lymphocytes Perhaps our findings therefore indicate that B lymphocytes in peripheral blood on these two occasions were immature

Two patients have been reported with familial or congenital pancytopenia and immune deficiency but they did not have congenital malformations or chromosome abnormalities (1 11) and thus did not have typical Fanconi s anemia

Immune deficiency is known to occur also in ataxia teleangiectasia (19) and Blooms syndrome (9) disorders with chromosome aberrations like those found in Fanconi's anemia Furthermore all three categories of patients are known to have an increased incidence of leukemia (22) It is tempting to speculate whether chromosome aberrations and immune deficiency are causally related and whether immune deficiency could possibly constitute the common condition for malignant growth in the three diseases

Transfer factor is a dialyzed leucocyte extract with a molecular weight below 10000 (21) In various situations it has been shown to transfer cellular immunity and thus improve the function of deficient T cells (10) As seen from Fig 3 the transfer factor injections in our patient were actually followed by improved T cell function Pentamidine and antibiotics given concomitantly could well be responsible for the clinical recovery from the pneumonitis Disappearance of postoperative immunosuppressive effects after splenectomy and cessation of antibiotics especially sulfametoxazole with trimetoprim may have played a role in improving T cell function A spontaneous improvement is also possible but the close time relation suggests an unspecific immunostimulating effect of the transfer factor therapy

In summary this report presents evidence of cellular immune deficiency suspected to be primary in a patient with Fanconi s anemia and furthermore suggests an association between transfer factor therapy and improvement of T cell function

ACKNOWLEDGEMENTS

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strup Laboratory of Paediatric Pathology Rigshospitalet for examining bone marrow and tracheal aspirates and C Petersen J F Kennedy Institute Glostrup Denmark for examining for sister chromatid exchanges

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Submitted Sept 9 1976
Accepted April 7 1977

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Acta Pædiatr Scand 66 753–756 1977

ASSESSMENT OF COR PULMONALE IN CYSTIC FIBROSIS BY ECHOCARDIOGRAPHY

E RYSSING

From the Ultrasound Laboratory and the Department of Paediatrics TG Rigshospitalet Copenhagen Denmark

ABSTRACT Ryssing E (Ultrasound Laboratory and Department of Paediatrics TG Rigshospitalet Copenhagen Denmark) Assessment of cor pulmonale in cystic fibrosis by echocardiography Acta Paediatr Scand 66 753 1977—Thirty-one patients with cystic fibrosis of varying severity were examined by echocardiography Right ventricular dimension (RVD) was above upper normal limit in 14 patients and right ventricular dimension index (RVD index) was higher than the upper normal limit in 11 patients Furthermore there was a significant relationship between increasing RVD index and 1) decreasing forced vital capacity (FVC) both actual test results and average 6 months values and 2) decreasing peak-expiratory flow rate (PEFR) both actual test results and average 6 months values This observation suggests a persistent heart involvement Five patients had either heart failure and/or electrocardiographic evidence of right ventricular abnormality These patients had increased RVD index and one patient with the highest RVD index died 8 weeks after the examination The present study has shown the usefulness of echocardiographic measurement of right ventricular dimension and of septal motion in assessing cor pulmonale before development of electrocardiographic abnormalities and right heart failure

KEY WORDS Echocardiography cor pulmonale cystic fibrosis

The purpose of the present investigation has been to study the development of cor pulmonale in cystic fibrosis (CF) by echocardiography There is only one publication on this subject (14)

MATERIAL AND METHODS

The material comprised 31 patients with CF of varying severity whose ages ranged from 4 to 23 years and averaged 1? 8 years None of the patients had previous signs of heart disease

Echocardiographic examinations were done using a Diasonograph NE 410 B with an unfocused 4 5 MHz transducer 10 mm in diameter a storage scope and Polaroid photographing of the screen picture Patients were examined in the supine position with head elevated to about 30° for the patient s comfort and to minimize coughing With the transducer placed along the left sternal border the location was sought which visualized in the best possible way the right ventricular anterior wall septum the posterior mitral valve and the posterior left ventricular wall Right ventricular dimension (RVD) was measured between the right septal surface and the right ventricular epicardium at the end diastole as determined from simultaneous electrocardiogram Because many patients had excessive excursions of the interventricular septum with respiration average values of at least 5 measurements but usually 10 to 12 measurements have been determined

Linear regression analysis was performed between the pulmonary function test results and the right ventricular dimension index (RVD index) which is the RVD divided by the body surface area and expressed in cm/m Septal motion was classified as normal (N) reversed (abnormal type A) or flattened (abnormal type B) (1) Abnormal septal motion occurred when the right ventricular cavity was dilated (2) Each patient underwent only one echocardiographic examination

Forced vital capacity (FVC) was measured using a McKesson Vitalor and peak-expiratory flow rate (PEFR) with a Wright anemometer Results were expressed as per cent of the predicted value based on the patient s height and sex The normal values for FVC given by Lyons et al (9) were used and for PEFR the normal values from this department were used (5)

Septal motion was classified as normal (N=3) abnormal type A (N=6) abnormal type B (N=1) and abnormal type A–B with pronounced respiratory fluctuations (N=1) in those 11 patient with RVD index above 2 2 Septal motion was classified as normal (N=13) abnormal type A (N=2) and abnormal type B (N=5) in those 20 patients with RVD index lower than 2 2 Abnormal septal motion was chiefly observed in patients with raised RVD index

Heart failure and electrocardiographic findings

Five patients had other signs of heart involvement in addition to raised RVD index These patients are lettered in the figures

Patient A Nineteen year old woman with periferal edema and enlargement of the liver but no extended neck veins ECG showed a right axis deviation RVD index was 2 5 and septal motion abnormal type A

Patient B Five years old girl with liver congestion and extended neck veins but no periferal edema ECG showed a right axis deviation P in lead II 2 5 mm and S in V_6 over 5 mm RVD index was 4 7 and septal motion abnormal type A She died 8 weeks later No autopsy

Patient C Seven year old boy without heart failure ECG showed a right axis deviation RVD index was 3 0 and septal motion abnormal type A–B with excessive respiratory fluctuations

Patient D Eight year old girl with slight distension of neck veins but no periferal edema or liver congestion ECG showed a right axis deviation and P in lead II 2 5–3 mm RVD index was 2 9 and septal motion was normal

Patient E Nine year old boy with enlargement of the liver and distended neck veins but no edema or electrocardiographic changes RVD index was 2 9 and septal motion abnormal type A

DISCUSSION

The echocardiographic investigation has demonstrated that patients with severe pulmonary changes have an abnormally increased RVD Furthermore there was a significant relationship between increasing RVD index and decreasing pulmonary function test results Because RVD is measured between the right ventricular epicardium and the right septal surface an increased RVD reflects right ventricular dilatation and/or hypertrophy Probably this suggests that these patients have cor pulmonale which is a wellknown complication to CF (3 11 15)

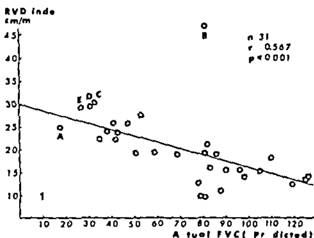
As an increase of RVD can also be obtained by a rotation of the heart bringing a larger part of the right ventricle within the ultrasonic beam serial measurements on the individual patient could of course be of greater value in predicting the existence of cor pulmonale

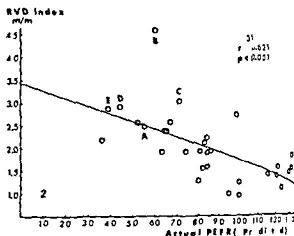
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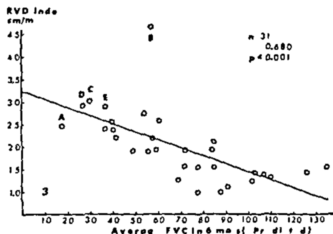
Rosenthal et al (14) found a significant correlation between *actual* pulmonary function test results and RVD index This finding is confirmed by our investigation with a still higher correlation coefficient Furthermore a highly significant correlation is found between average six months values for pulmonary function test and RVD index suggesting a persistent heart involvement

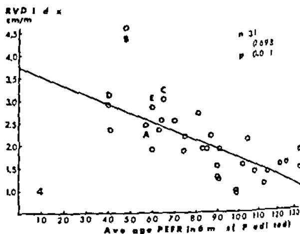
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Lundstrom et al (8) found a fairly good relationship between increased RVD and right ventricular size based on the angiogram Increased RVD is found chiefly in patients with right ventricular volume overload (1 2 6 7 10 13 16 17) but also in some patients with pressure overload (1 4 6 7)









Figs. 1–4. Increasing right ventricular dimension index correlates significantly with decreasing actual forced vital capacity (Fig. 1), actual peak expiratory flow rate (Fig. 2), average forced vital capacity in 6 months (Fig. 3) and average peak expiratory flow rate in 6 months (Fig. 4). A–E represent patients with other heart symptoms. See text. RVD index=right ventricular dimension index. FVC=forced vital capacity. PEFR=peak expiratory flow rate.

RESULTS

Echocardiographic findings

Thirty one patients with body surface areas above 0.65 m² had RVD values between 1.4 and 4.3 cm (average 2.3 cm). Thirty controls with body surface areas above 0.65 m² too had RVD values between 1.3 and 2.2 cm (average 1.6 cm). RVD of 14 patients were found higher than the upper normal limit.

In the control group RVD index varied between 1.0 and 2.2 cm/m² and in the patient group between 1.0 and 4.7 cm/m². Eleven patients had RVD index above the upper normal limit.

Increasing RVD index correlated significantly with decreasing actual FVC ($r=-0.567$, $p<0.001$) and actual PEFR ($r=-0.621$, $p<0.001$) (Figs. 1 and 2).

Furthermore, RVD index was related to average values in 6 months for FVC and PEFR with the purpose to determine whether the finding of raised RVD index was caused by an acute or by a persistent decrease in pulmonary function. As our patients are frequently seen in the outpatient clinic 6 to 19 pulmonary function test results over a 6 month period in each patient are available. Figs. 3 and 4 show a highly significant correlation between increasing RVD index and decreasing average 6 month values for FVC ($r=-0.680$, $p<0.001$) and for PEFR ($r=-0.698$, $p<0.001$). This suggest a persistent heart involvement as a result of chronic pulmonary alterations.

Septal motion was classified as normal (N= 3) abnormal type A (N=6) abnormal type B (N=1) and abnormal type A–B with pronounced respiratory fluctuations (N=1) in those 11 patient with RVD index above 2 2 Septal motion was classified as normal (N= 13) abnormal type A (N=2) and abnormal type B (N=5) in those 20 patients with RVD index lower than 2 2 Abnormal septal motion was chiefly observed in patients with raised RVD index

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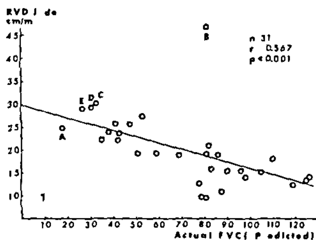
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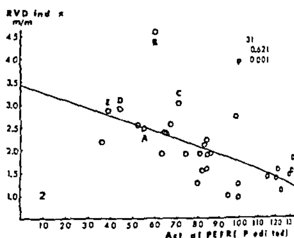
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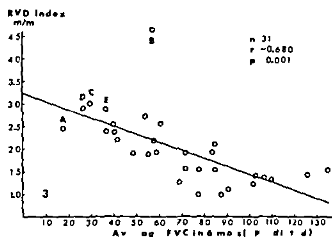
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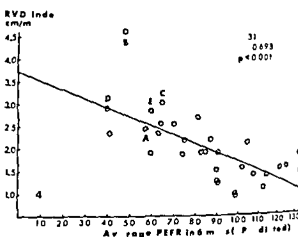
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Figs 1–4 Increasing right ventricular dimension index correlates significantly with decreasing actual forced vital capacity (Fig 1) actual peak expiratory flow rate (Fig 2) average forced vital capacity in 6 months (Fig 3) and average peak expiratory flow rate in 6 months (Fig 4) A–E represent patients with other heart symptoms See text RVD index=right ventricular dimension index FVC=forced vital capacity PEFR=peak-expiratory flow rate

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Submitted Febr 1 1977
Accepted April 23 1977

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Acta Paediatr Scand 66 757–759 1977

BILIRUBIN INHIBITS HEXOSE MONOPHOSPHATE SHUNT ACTIVITY OF PHAGOCYTOSING NEUTROPHILS

Y H THONG and V RENCIS

From the Department of Paediatrics University of Adelaide Adelaide Children s Hospital North Adelaide South Australia Australia

ABSTRACT Thong Y H and Rencis V (Department of Paediatrics University of Adelaide Adelaide Children s Hospital North Adelaide 5006 South Australia Australia) Bilirubin inhibits hexose monophosphate shunt activity of phagocytosing neutrophils Acta Paediatr Scand 66 757 1977 —Neutrophil hexose monophosphate (H M P) shunt activity was measured by the conversion of 14C 1 glucose to $^{1}CO_2$ during ingestion of polystyrene latex particles Unconjugated bilirubin at the concentration of 1×10^{5} M was found to markedly inhibit H M P shunt activity Since H M P shunt activity of neutrophils is an important prerequisite of microbicidal function it is suggested that jaundiced newborns may be more susceptible to bacterial infections

KEY WORDS Bilirubin neutrophils hexose monophosphate shunt

Although the extraneural toxicity of unconjugated bilirubin has been known for many years (13 4) the adverse effects on the immune system have only recently been recognised Ansaldi et al (1) reported lower immunoglobulin levels in infants with neonatal jaundice Others have found impaired antibody production following immunization with diphtheria pertusis tetanus (11) and measles (7) Inhibition of lymphocyte responsiveness to phytohaemagglutinin stimulation has also been noted (6 15) We have studied the effect of unconjugated bilirubin on neutrophil function as measured by glucose oxidation via the hexose monophosphate (H M P) shunt during phagocytosis of latex particles (3)

MATERIALS AND METHODS

Polymorphonuclear leukocytes from healthy donors were purified by centrifugation 30 min at 00 G on a hypaque ficoll gradient S G 1095 Cells were washed twice and resuspended in glucose free Earl s solution Viability was assessed by trypan blue dye exclusion at the beginning and end of experiments Experiments were performed in Warburg flasks in 1 ml volumes (3) Stimulated flasks contained ?–4×10^{6} cells ml^{-1} 1 5×10 ^{8} M C 1 glucose and 0 1 ml of 1 3 dilution of 0 80 μ size polystyren latex particles Latex particles were omitted from unstimulated flasks Test flasks contained appropriate concentrations of unconjugated bilirubin solubilized in 10^{-4} M sodium carbonate Control flasks contained similar concentrations of the solvent only Flasks were incubated for 45 min on a shaking water bath at 37°C the $^{1}CO_2$ captured by 0 1 ml of 5 N NaOH in the centre well and counted in a Packard tricarb scintillation counter Inhibition of H M P shunt activity was calculated as follows

% inhibition=

$$100 - \frac{\text{cpm stimulated containing bilirubin} - \text{cpm unstimulated}}{\text{cpm stimulated without bilirubin} - \text{cpm unstimulated}} \times 100$$

In order to determine whether phagocytosis of latex particles was affected by bilirubin a separate set of experiments were performed as described above except that at the end of the incubation period the cells were placed on glass slides and the number of particles per 100 cells determined by microscopic observation

Further analysis of the relationship between clinical condition and RVD index is not performed because we don t employ clinical scoring However, the five lettered patients with other signs of heart involvement have increased RVD index and the highest RVD index was determined in patient B who died 8 weeks after the examination

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Submitted Febr 21 1977
Accepted April 19 1977

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Table 1 *Effect of bilirubin on H M P shunt activity of phagocytosing neutrophils*

Bilirubin concentration	% Inhibition (Mean ± S E of 5 experiments)	Statistical difference compared to control (t test)
0 (control)	0	–
1×10^{-5} M	51±7	$p<0.01$
5×10^{-6} M	23±8	$p>0.05$
1×10^{-6} M	10±3	$p>0.05$

Table 2 *Effect of albumin on bilirubin induced suppression of H M P shunt activity*

Bilirubin concentration	Albumin concentration	% Inhibition (Mean ± S E of 5 experiments)
0 (control)	0	0
10^{-5} M	0	63±5[a]
10^{-5} M	10^{-5} M	11±6[b]
10^{-5} M	10^{-6} M	27±11[b]

[a] Inhibition by 10^{-5} M bilirubin of 63±5% is not significantly different from 51±7% obtained in the first set of experiments (Unpaired t test $p>0.2$)
[b] Significant reversal of bilirubin induced suppression of H M P shunt activity by 10^{-5} M albumin ($p<0.01$) but not by 10^{-6} M albumin ($p>0.05$)

RESULTS

Phagocytosis of latex particles did not appear to be affected by the presence of unconjugated bilirubin The number of particles phagocytosed per 100 cells were 124 8±12 4 (mean± S E of 6 experiments) in the presence of 1×10^{-5} M bilirubin compared to 115 3×10 9 in controls ($p>0.6$)

Basal H M P shunt activity was also unaffected by the concentrations of bilirubin used in these experiments In contrast there was marked inhibition of H M P shunt activity of 51±7% on stimulated neutrophils at bilirubin concentration of 10^{-5} M (Table 1) In a separate set of experiments it was also found that equimolar concentrations of albumin reversed the inhibitory effect of bilirubin (Table 2)

DISCUSSION

During phagocytosis there is a marked increase in glucose oxidation via the hexose monophosphate shunt resulting in the production of hydrogen peroxide and superoxide important chemical mediators of microbial killing (9) Unconjugated bilirubin is known to affect a number of cellular enzyme systems (5 10 12 18) but the mechanism by which bilirubin inhibits neutrophil H M P shunt activity is unclear One explanation may be its known effect on the enzyme N A D H oxidase (5) This enzyme located primarily on the plasma membrane of neutrophils is deficient in chronic granulomatous disease (2 16) In the normal sequence of events the plasma membrane is invaginated to form the phagosome N A D H oxidase is activated hydrogen peroxide diffuses through the cytoplasm which in turn stimulates H M P shunt activity (9)

The clinical significance of our findings is difficult to evaluate The concentration of bilirubin (10^{-5} M) which affects neutrophil function in vitro corresponds to a serum level of 0 6 mg per 100 ml and is within the physiologic range Its tight binding to albumin (14) would prevent a deleterious effect on cells However in clinical states of hyperbilirubinaemia, albumin binding capacity can be saturated and neutrophil function impaired This may in part account for the observation that patients with congenital atresia of the bile ducts where a substantial fraction of serum bilirubin may be of the unconjugated variety have an increased susceptibility to intercurrent infection (17) Moreover jaundice is the second most common clinical feature associated with neonatal sepsis (8) Although neonatal hyperbilirubinaemia is generally regarded as a consequence of sepsis rather than a predisposing cause clinical studies to clarify this issue may prove rewarding

ACKNOWLEDGEMENTS

Supported in part by the Adelaide Children s Hospital Research Trust We wish to thank Professor George Maxwell for stimulating discussion and Geoffrey Harvey and Dietmar Ness for assistance

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Acta Pædiatr Scand 66 761–765 1977

THE OCCURRENCE AND ORIGIN OF DDT IN HUMAN MILK

ERKKI VUORI HILKKA TYLLINEN PEKKA KUITUNEN and AILA PAGANUS

From the Milk Bank of the Children s Hospital the Department of Public Health Science University of Helsinki and the State Veterinary Medical Institute Helsinki Finland

ABSTRACT Vuori E Tyllinen H Kuitunen P and Paganus A (The Milk Bank of the Children s Hospital the Department of Public Health Science University of Helsinki and the State Veterinary Medical Institute Helsinki Finland) The occurrence and origin of DDT in human milk Acta Paediatr Scand 66 761 1977.—Gas chromatography has been applied for the analysis of organochlorine compounds of 49 samples of human milk The average total DDT (2,2 bis(4-chlorophenyl)1 1 1 trichloroethane) content in human milk was found to be 0 058 mg/kg ranging from 0 017–0 17 mg/kg (1 57 mg/kg milk fat with a range of 0 50–4 00 mg/kg) Thirty four cases contained traces of dieldrin but the content of dieldrin reached 0 008 mg/kg in only one milk sample The average content of PCB (polychlorinated biphenyls) was 0 024 mg/kg of human milk with a range of 0 011–0 054 mg/kg (0 65 mg/kg of milk fat with a range of 0 33–1 10 mg/kg) The ratio of DDT meta bolites/DDT varied from 1 1 to 7 8 (mean 2 8) Studies were also made of the effect of the weight weight loss diet smoking habits and parity of the nursing mother upon the content of organochlorine compounds in human milk A significant positive correlation was observed between the DDT content of human milk fat and cigarette smoking

The average daily intake of total DDT for Finnish breastfed babies was calculated to be 0 0093 mg/kg 1 9 times more than the daily intake of 0 005 mg/kg indicated by FAO/WHO as the acceptable value

KEY WORDS Insecticides polychlorobiphenyl compounds human milk

The discovery of DDT in human milk (7) has been followed by a number of reports upon the harmful action of DDT in the biosphere (13) Published information of this type has led to a ban being imposed on the use of DDT as a pesticide in many countries In Finland the use of DDT has been restricted since 1971 and completely forbidden with effect from January 1 1977 Some researchers have looked for factors which influence the DDT content of human milk Studies have included food stuffs (3 15) the domestic use of pesticides (3 8 15) the parity (3 6) and weight of the nursing mother (5) and recently cigarette smoking (3) What determines the DDT levels in human milk in a country where the use of DDT has been restricted for years? With a view to the solution of this problem analyses have been made of the content of organochlorine compounds (OCC) of 49 human milk samples along with an investigation of the possible sources of DDT in human beings

MATERIALS AND METHODS

Random collections were made of 49 individual human milk samples during the period November 1973 to March 1974 from donors of the Milk Bank of the Children s Hospital University of Helsinki All of the donors were living in the Helsinki metropolitan area To each one there

Abbreviation

DDT=? ? bis(4-chlorophenyl)1 1 1 trichloroethane
DDE=2 ? bis(4-chlorophenyl)1 1-dichloroethylene
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total DDT=DDT+DDE+DDD
PCB=polychlorinated biphenyls
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Table 1 *Age weight weight loss (post partum) phase of lactation of the donor group fat concentration and the average amount of milk donated daily*

Fat concentration 49 observations otherwise 46

	Mean	S D	Range
Age (years)	26 3	3 4	20–35
Weight (kg)	65 0	11 9	46–112
Weight loss (kg/month)	2 0	1 5	0 0–7 5
Phase of lactation (days)	41 4	33 7	10–152
Fat concentration (%)	3 7	0 9	2 2–7 1
Milk donated (ml/day)	351	321	45–1 975

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The samples of human milk were weighed and dried with anhydrous sodium sulphate The fat was extracted and the OCC were purified by thin layer chromatography The analyses were carried out by gas chromatography To ensure the accuracy of identification the analyses were performed twice with two different types of columns The degree of purity of the standards for the determinations of DDT DDE DDD and dieldrin was 99% (Analytical Standards Ltd Sweden) The standards for the determination of PCB were prepared from a technical grade product (Clophen A 60) with a chlorine content of 60% A detailed account of the method has been published earlier (12) The analyses were carried out in the State Veterinary Medical Institute

RESULTS

The mean age of the donors was 26 3 years the mean phase of lactation was 41 4 days post partum and the mean fat concentration

Table 2 *Dietary habits of the donors*

The numbers indicate percentages of mothers in each dietary habits group

	More than 3 times a week	Only 1–2 times a week	Only 1–2 times a month	No
Meat/meat products	91 3	8 7	–	–
Fish	4 3	69 6	23 9	2 2
Liver	2 2	71 7	19 6	6 5

3 7% (Table 1) Almost every mother ate meat or meat products daily and the majority of the mothers ate fish and liver 1–2 times a week (Table 2) Only 8 7% of the donors had a daily fat intake of more than 100 g and 47 8% from 50 g to 100 g Forty of the 49 mothers provided answers to the questions in regard to smoking habits 62 5% of the mothers had never smoked, 20 0% had smoked cigarettes for 1–3 years 12 5% for 3–5 years 2 5% for 5–10 years and 2 5% for more than 10 years The majority of the mothers (65 2%) were nursing their first infant 32 6% their second and 2 2% their third

Table 3 lists the concentrations of DDT total DDT and PCB found in human milk and milk fat The mean ratio of DDT metabolites (=DDE+DDD) to DDT was 2 8 with a range of from 1 1 to 7 8 Traces of dieldrin were discernible in 34 cases although the concentration of dieldrin attained 0 008 mg/kg human milk in only one sample Fig 1 illustrates the distribution of different levels of total DDT in

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	Mean	S D	Range	Mean	S D	Range
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Total DDT	0 058	0 032	0 017–0 17	1 57	0 75	0 50–4 00
PCB	0 024	0 008	0 011–0 054	0 65	0 17	0 33–1 10

[a] Under 0 005 mg/kg

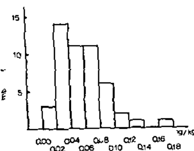


Fig 1 Distribution of different levels of total DDT in human milk

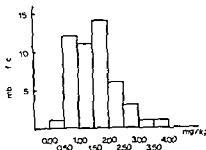


Fig 2 Distribution of different levels of total DDT in human milk fat

human milk and Fig 2 the corresponding distribution for human milk fat

A significant correlation was observed between cigarette smoking and the concentration of DDT and an almost significant correlation between cigarette smoking and the total DDT concentration as is evident from Table 4 No significant correlation was apparent between the dietary habits investigated and the concentrations of DDT total DDT and PCB Moreover no significant correlations were observable between the concentrations of OCC and the age the phase of lactation amount of donated milk parity weight and weight loss of the donors

DISCUSSION

In comparison with other European countries the level of OCC of human milk is low in Finland (Table 5) It can be seen that the total DDT concentration in human milk has diminished in Sweden during recent years whereas the concentration of PCB has increased A diminution in DDT levels following the restriction of its use has also been evident elsewhere (5) The low levels of DDT in Finland are understandable as the use of DDT has never been extensive in this country during the period 1946–1971 sales of DDT here amounted to a total of 304 tons (9)

Kroger (6) has found that the concentrations of DDT in the milk fat of mothers who had nursed three or more babies were below average The results obtained by Wilson et al (15) demonstrated a fall in DDT levels with increasing maternal age although the findings of Knoll & Jayaraman (5) were directly opposed to this Recently Bradt & Herrenkohl (3) have found that the number of children nursed accounted statistically for 21 % of the variance in total DDT They also calculated that during nursing an average American mother excretes twice the mean daily intake of DDT In the present study no significant correlation was apparent between maternal age or parity and the OCC levels of human milk This may be explained by the fact that the majority of the mothers were nursing their first infant and only one mother her third Wilson et al (15) have also studied the dietary habits of mothers and they found no significant correlations between the total DDT concentrations in

Table 4 *Correlation matrix of the concentrations of DDT total DDT and PCB on basis of milk fat and cigarette smoking*

Cigarette smoking 40 observations otherwise 49 Lacking observations replaced by mean values

	DDT	Total DDT	PCB
Total DDT	756		
PCB	437[a]	501	
Cigarette smoking	385[b]	362	09[d]

Nearly significant 0 05 > p > 0 01
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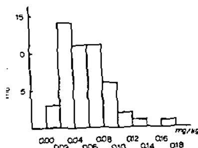


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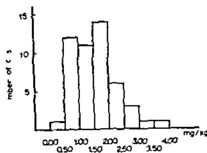


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PCB	*0 024*	0 008	0 011–0 054	0 65	0 17	0 33–1 10

[a] Under 0 005 mg/kg

3 Wasserman M Tomatıs L & Wasserman D Storage map of organo-chlorine compounds (OCC) ın humans *International Symposium Proceedings Recent Ad ances ın the Assessment of the Health Effects of Environmental Pollution II* 1053 1975
4 Westoo G & Noren K Halter av klorpesticider och polyklorerade bifenyler ı svensk modersmjolk *Var foda 24* 41 197?
5 Wilson D J Locker D J Ritzen A Watson J T & Schaffner W DDT concentrations ın human mılk *Am J Dıs Child 125* 814 1973
16 WHO Pesticide residues ın food *Technical Report Series No 502* Geneva 197

Submitted Jan 3 1977
Accepted March ?4 1977

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Table 5 *Average total DDT and PCB concentrations in human milk in some European countries*

Country	Year	Total DDT (mg/kg)	PCB (mg/kg)	Ref
England	1963–64	0 128	–	4
France	1972–73	0 130	–	8
GDR	1971	0 370	–	5
GFR	1973–74	0 107	0 10	11
Norway	1969–70	0 056–0 110	0 011	2
	1975	0 082	–	1
Sweden	1967	0 11	0 014	14
	1968–69	0 088	0 018	14
	1971–72	0 086	0 025	14
Finland[b]	1973–74	0 058	0 024	–

The authors give a value of 3 24 mg/kg of milk *fat* The value in the table has been calculated on the assumption that the fat concentration of human milk averages 4%
[b] Present study

milk and the habit of eating fish or meat Our results are in agreement with this observation

Knoll & Jayaraman (5) found that the milk of obese women contained lower amounts of OCC than did that of women of normal weight In the present study no correlation was observable between the OCC content of milk and the weight or post partum weight loss of the donor

Bradt & Herrenkohl (3) have shown that the mean total DDT for non smokers was lower than for cigarette smokers 15% of the variance of total DDT was explicable by the number of cigarettes smoked daily In this study it became evident that cigarette smoking showed a significant correlation with the DDT concentrations in milk fat These correlations are understood when one considers that tobacco plants are regularly treated with DDT (10) It is obvious that in Finland where DDT has not been used for years a large proportion of DDT comes from cigarettes this is further supported by the finding that cigarette smoking has a closer correlation with the DDT than with the total DDT (including the metabolites of DDT) content of milk fat (Table 4)

According to the calculations of Westoo & Noren (14) a Finnish infant weighing 5 kg who consumes 800 g of milk a day would receive an average daily dose of 0 0093 mg/kg of total DDT Although this is among the lowest values in the world it is nevertheless almost twice as much as ADI value of 0 005 mg/kg indicated by FAO/WHO (16)

Although at present the OCC residues in human milk do not offer any threat to public health in Finland we must agree with the statement by Wasserman et al (13) that The greatest exposure of the general population nonoccupationally exposed to OCC is that of infants fed by mother s milk This fact in conjunction with the marked positive correlation between cigarette smoking and the DDT content of human milk provides further reason to advise women not to smoke

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Acta Pædiatr Scand 66 767–775 1977

REVIEW ARTICLE

DRUG USAGE AND ADVERSE DRUG REACTIONS IN PAEDIATRIC PATIENTS

J WHYTE and E GREENAN

From the University Department of Child Health and the Royal Hospital for Sick Children Yorkhill Glasgow Scotland

ABSTRACT Whyte J and Greenan E (University Department of Child Health Royal Hospital for Sick Children Glasgow Scotland) Drug usage and adverse drug reactions in paediatric patients Acta Paediatr Scand 66 767 1977 —The development of epidemiological methods for the study of adverse drug reactions is reviewed in connection with the presentation of data obtained by intensive monitoring of 1 000 admissions to a medical paediatric unit Compared with adults and American children the patients received fewer drugs and experienced fewer reactions while in hospital The drug usage pattern was different from that of American paediatric practice and general practice in the United Kingdom Fifty-one (6%) patients experienced 119 adverse drug reactions These occurred more frequently in children suffering from serious disorders and in the majority of cases the basic therapy was continued regardless of the severity of the drug side effects Treatment was required for the effects of 66 (55%) adverse reactions It appears that drug monitoring in paediatric practice may be of greater value if surveillance programmes are designed to provide a therapeutic audit and extended to include children receiving drugs in the community

KEY WORDS Drug usage drug surveillance adverse drug reactions drug monitor

Concern about the hazards of drug therapy has grown with the increasing availability of potent medicines and has led to the introduction of methods designed to investigate the prevalence nature and significance of adverse drug reactions (21)

Following the thalidomide disaster of the early 1960 s spontaneous reporting of adverse reactions was encouraged by the development of central registries which collated the reports and instituted further enquiries The increased volume of information obtained in this way has enabled these agencies to publish comprehensive lists of the side-effects of many drugs but the continuing failure of practitioners to report many of the adverse reactions which they encounter has prevented the registries from assessing the frequency with which drug induced disorders occur in the community (22)

The potential value of intensive drug monitoring in hospitals was demonstrated by pilot studies in several centres (6 18 19 25) and much valuable information has been obtained from event orientated and drug orientated surveys conducted in the hospital setting (3 16) Coull and his co workers have demonstrated the particular value of the latter approach used in conjunction with a regional drug information system and have described the detailed investigation of suspected drug reactions (3 4) It is apparent however that this method has been of greatest value when used to test pre existing hypotheses about drug effect associations and that it cannot by the nature of its design act as an alerting system Similarly it is unable to measure the prevalence of iatrogenic disease in a hospital population exposed to many drugs

Table 1 *Drug usage in paediatric patients*

Age group (years)	No of patient admissions	No not on therapy while in hospital	No of drugs prescribed in hospital	Average per patient
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12+	55	3	234	4 3
Total	932	249	2 179	2 3

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Analysis of factors predisposing to the development of adverse reactions

Statistical analysis was carried out to test the significance of factors predisposing to adverse drug reactions As in other studies only reactions which were definite or probable were included When testing the relationships between drug reactions and the number of drugs administered only admission episodes complicated by reactions to hospital therapy were included as data on pre admission drug usage were unreliable for reasons already reported (23)

The number of patients experiencing adverse reactions was too small to permit standardisation for all of the variants

RESULTS

Population and drug usage

During the survey period 932 admission episodes involving 844 children (499 males) were studied The average duration of stay in the unit was 8 9 days younger children predominated (Table 1) and respiratory infection was the most frequently recorded diagnosis (Table 2) Accidental ingestion of foreign substances caused the admission of 52 patients 35 of whom were poisoned by drugs not in current use by the affected children

Over the ten month period of the study 2 179 *prescriptions were recorded* an average of 2 3 drugs per patient admission or 0 26 per patient per day (usage corrected for duration of stay) Two hundred and forty nine (26 7%) patients did not receive drugs after admission and this occurred most frequently in children

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E N T system	19
Others	80
Total	932

Patient orientated comprehensive drug surveillance has not been limited in these ways and has been used successfully in many centres throughout the world (11) In studies which have used this method the clinical progress of a cohort of patients admitted to a surveyed area (e g a medical ward) has been monitored by doctors pharmacists or nurses (5 7 8 19) to obtain details of drug usage (denominator data) and adverse drug reactions (numerator data) The data obtained have allowed more accurate measurement of the incidence of adverse drug effects and have enabled workers to delineate groups of patients at particular risk e g the elderly (7) Also when applied on a large scale co operative basis the method has successfully detected previously unsuspected drug interactions and reports have suggested that it may be of use for the assessment of drug efficacy (9 20)

Similar work in paediatrics has been on a smaller scale but has highlighted the differences which exist between the drug usage and adverse drug reactions of children and adults (2 13) However by adopting a purely epidemiological approach these studies have failed to relate the clinical effects of drug induced disorders to the severity and prognoses of the diseases under treatment and have not allowed the clinician to assess their significance in relation to his own therapeutic practice

This paper describes our experience with a comprehensive drug surveillance programme carried out to obtain data for comparison with other published results and to assess the clinical importance of adverse drug effects in the therapeutic setting in which they occurred

METHOD

Successive admissions to a medical paediatric unit (48 beds) with regional responsibilities for cardiac investigation and the treatment of leukaemia were intensively monitored using a method similar to that of Slone and his co-workers (19) One thousand admission episodes involving 909 children occurred over a 10 month period but 68 were excluded from the study when the children required general anaesthetic or were transferred to an other ward

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Definitions and classifications

An adverse reaction was defined as any undesired or unintended response to the patient s own current medication, thus excluding accidental poisoning by other drugs or substances

Probability of adverse drug reactions

Reactions were classified as

Definite directly attributable to a drug having a clear temporal relationship to the time of administration and confirmed by positive rechallenge or laboratory investigation e g evidence of abnormal blood levels etc

Probable occurring with a clear temporal relationship to administration and improving on withdrawal of therapy

Possible having some temporal relationship to administration but the effects could have been due to the basic illness

Severity of adverse reactions

Reaction severity was classified as

1 Causing death
2 Life threatening—demanding immediate action to prevent death
3 Severe—necessitating the use of supportive therapy prolonging the patient s admission by 3 or more days or causing admission to hospital and resulting in either of the above
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To provide a classification of the mechanisms of adverse drug reactions which could be readily interpreted in the clinical situation a modification of schemes suggested by several authors was adopted (5 7 17 18)

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This paper describes our experience with a comprehensive drug surveillance programme carried out to obtain data for comparison with other published results and to assess the clinical importance of adverse drug effects in the therapeutic setting in which they occurred

METHOD

Successive admissions to a medical paediatric unit (48 beds) with regional responsibilities for cardiac investigation and the treatment of leukaemia were intensively monitored using a method similar to that of Slone and his co-workers (19) One thousand admission episodes involving 909 children occurred over a 10 month period but 68 were excluded from the study when the children required general anaesthetic or were transferred to an other ward

A research nurse (E G) acting as the drug monitor interviewed parents to obtain details of pre admission therapy and previous adverse drug reactions A record of each child s age sex weight height diagnosis and duration of stay in hospital was also completed By visiting the wards daily examining medical and nursing records and attending clinical ward rounds the monitor detected adverse events which might have been due to drug therapy When a suspected adverse drug reaction was reported the study team together with the physician in charge of the patient reviewed the circumstances to decide if a relationship existed between the adverse event and the drugs administered Adverse reactions detected in this way were then studied further to determine their nature severity and mechanisms and their effects on the clinical management of the patient Data obtained were recorded on standard forms and entered on a computer file for analysis

Definitions and classifications

An adverse reaction was defined as any undesired or unintended response to the patient s own current medication thus excluding accidental poisoning by other drugs or substances

Probability of adverse drug reactions

Reactions were classified as

Definite directly attributable to a drug having a clear temporal relationship to the time of administration and confirmed by positive rechallenge or laboratory investigation e g evidence of abnormal blood levels etc

Probable occurring with a clear temporal relationship to administration and improving on withdrawal of therapy

Possible having some temporal relationship to administration but the effects could have been due to the basic illness

Severity of adverse reactions

Reaction severity was classified as

1 Causing death
2 Life threatening—demanding immediate action to prevent death
3 Severe—necessitating the use of supportive therapy prolonging the patient s admission by 3 or more days or causing admission to hospital and resulting in either of the above
4 Moderate—necessitating the administration of an antidote or symptomatic treatment prolonging admission by 1–3 days or causing admission and resulting in either of the above
5 Mild—incidental in the patient s progress and not requiring treatment

Mechanisms of adverse reactions

To provide a classification of the mechanisms of adverse drug reactions which could be readily interpreted in the clinical situation a modification of schemes suggested by several authors was adopted (5 7 17 18)

Reactions were classified as

1 Intended effect excessive (TOXIC) (a) with overdosage (according to Clark s rule) (b) without overdosage (c) potentiation—possible additive effect of drugs with similar actions

Table 1 *Drug usage in paediatric patients*

Age group (years)	No of patient admissions	No not on therapy while in hospital	No of drugs prescribed in hospital	Average per patient
0–1	219	79	434	2.0
1–2	176	48	258	2.0
2–3	97	35	184	1.9
3–4	86	23	184	2.1
4–5	65	11	189	3.0
5–6	53	12	117	2.2
6–7	46	5	137	3.0
7–8	45	5	109	2.4
8–9	38	7	80	2.1
9–10	39	9	115	3.0
10–11	29	7	62	2.1
11–12	34	5	76	2.3
12+	55	3	234	4.3
Total	932	249	2 179	2.3

2 Unintended but pharmacologically predictable side effects (*SIDE EFFECTS*) (*a*) with overdosage (*b*) without overdosage (*c*) potentiation
3 Allergic—showing features suggestive of allergy e.g. rash angio-oedema anaphylaxis
4 Secondary effect—indirectly attributable to the action of a drug
5 Irritant—causing irritation of epithelial surfaces
6 Mechanism unknown

Analysis of factors predisposing to the development of adverse reactions

Statistical analysis was carried out to test the significance of factors predisposing to adverse drug reactions. As in other studies only reactions which were definite or probable were included. When testing the relationships between drug reactions and the number of drugs administered only admission episodes complicated by reactions to hospital therapy were included as data on pre-admission drug usage were unreliable for reasons already reported (23).

The number of patients experiencing adverse reactions was too small to permit standardisation for all of the variants.

RESULTS

Population and drug usage

During the survey period 932 admission episodes involving 844 children (499 males) were studied. The average duration of stay in the unit was 8.9 days; younger children predominated (Table 1) and respiratory infection was the most frequently recorded diagnosis (Table 2). Accidental ingestion of foreign substances caused the admission of 52 patients, 35 of whom were poisoned by drugs not in current use by the affected children.

Over the ten month period of the study 2 179 prescriptions were recorded, an average of 2.3 drugs per patient admission or 0.26 per patient per day (usage corrected for duration of stay). Two hundred and forty nine (26.7%) patients did not receive drugs after admission and this occurred most frequently in children

Table 2 *Most frequently recorded primary discharge diagnoses*

Diagnosis	Number of cases
Congenital heart disease	72
Febrile convulsion	68
Upper respiratory infection	49
Asthma	41
Pneumonia/bronchopneumonia	36
Bronchitis	30
Other diagnoses (by system)	
Central nervous system	100
Blood and lymphatic system	80
Uro-genital system	69
Alimentary system	63
Respiratory system	56
Endocrine system	53
Ingestion and poisoning	52
Skin disorders	39
Nutritional disorders	25
E N T system	19
Others	80
Total	932

Table 3 *Most frequently prescribed drugs*

Drug	No of pre scrip-tions	% of total pre scrip-tions
Phenobarbitone	87	3 9
Benzylpenicillin	70	3 2
Dextrose/saline infusion fluid	70	3 2
Phenoxymethylpenicillin	67	3 1
Sodium iothalamate	*55*	*2 5*
Other drugs (by pharmacological group)		
Antibacterials	332	15 2
Anticonvulsant/sedatives	163	7 5
Radiodiagnostic agents	146	6 7
Hypnotics sedatives and anti convulsants	135	6 2
Analgesics and antitussives	103	4 7
Corticosteroids	102	4 6
Skin ointments and applica tions	95	4 4
Intravenous infusion fluids	79	3 6
Drugs acting on the autonomic system	77	3 5
Others	598	27 4
Total	2 179	100

Table 4 *Pre admission drug usage (590 patients)*

Types of drugs	Number of drugs taken	% of total
Antibacterials	195	20 3
Analgesics (simple)	122	12 7
Skin preparations	81	8 5
Compound antihistamine prepara tions	56	5 8
Antihistamines	49	5 0
Anticonvulsants	37	3 9
Broncho dilators	37	3 9
Unidentified	103	10 7
Others	281	29 3
Total	961	100

who suffered from viral respiratory infection and febrile convulsions Drug use was greatest in the oldest age group due to the relative fre quency of cardiac investigation and the treat *ment of malignancy in this group*

Phenobarbitone was the most commonly used drug (Table 3) but preparations with anti bacterial activity constituted the largest phar macological group comprising 469 (21 5%) of the prescriptions Anticonvulsant/sedatives were the next largest group and were followed in frequency by radiodiagnostic agents

The deployment of drugs for different pur poses in differing situations explains the rela tive frequency of barbiturate usage as these drugs were popular as basal sedatives for in vestigative procedures Five hundred and forty (24 7%) prescriptions were for drugs given to aid investigations and 108 treated patients received drugs for this purpose alone Thus of the total population 357 (38 3%) patients did not receive drug therapy directed at the cure or symptomatic relief of the disease causing admission

It was apparent that the pattern of prescrib *ing of British hospital practice differed from* that of general practice Table 4 illustrates the pre admission medication of a comparable group of children admitted to the same unit

Prevalence of adverse drug reactions

Adverse drug reactions were detected in 51 (6%) patients Thirty nine (6 5%) of 595 chil dren who received medication in hospital ex perienced at least one adverse reaction to therapy while 13 patients had reactions to

Table 5 *Relationship of adverse reactions to time of detection and source of prescription*

	Reactions causing admission	Reactions present at admission	Reactions detected in hospital due to prior therapy	Reactions due to hospital therapy
Prescribed by general practi tioner	8 (8)	1 (1)	4 (4)	0
Prescribed by hospital staff	4 (4)	12 (3)	0	90 (39)
Total	12 (12)	13 (4)	4 (4)	90 (39)

Number of patients involved in parenthesis

Table 6 *Nature of 119 adverse reactions*

System	No	Manifestation	No
Gastro-intestinal	39	Vomiting	25
		Monilial infection	7
		Diarrhoea	2
		Anorexia	1
		Abdominal pain	1
		Constipation	1
		Melaena	1
		Stomatitis	1
Haematological	32	Bone marrow depression	3
Cutaneous	0	Rash (maculo-papular)	7
		Urticaria	4
		Alopecia	2
		Rash (erythematous)	2
		Cushingoid facies	2
		Inflamed injection site	1
		Angioneurotic oedema	1
		Stevens Johnson syndrome	1
Neuro-muscular	14	Somnolence	8
		Ataxia	2
		Dystonia	2
		Stupor	1
		Lethargy	1
Metabolic	6	Hypokalaemia	2
		Hypoglycaemia	2
		Raised blood urea	1
		Raised SGOT	1
Cardio-vascular	5	Anaphylactic reaction	2
		Bradycardia	1
		Hypotension	1
		Hypotension (postural)	1
Respiratory	2	Pneumonia	2
Other	1	Fever	1
Total	119		119

drugs prescribed by their general practitioners (Table 5) Twelve (10%) reactions caused admission to hospital and affected 12 patients The 51 affected patients experienced 119 adverse reactions of which 28 were definite 78 were probable and 13 were possible

Nature of adverse drug reactions (Table 6)

Gastro intestinal symptoms were the most frequent manifestations of adverse drug effects and together with skin disorders constituted 49% of the total The large number of haematological abnormalities is due to the frequency with which reactions occurred in patients receiving anti neoplastic therapy and the repeated laboratory tests which were performed on this group However these patients also exhibited clinically detectable reactions more frequently than did others

Mechanisms of adverse drug reactions (Table 7)

Unintended but predictable side effects were the most frequent adverse reactions to hospital therapy Allergic responses to drugs occurred with a frequency comparable to that recorded in other studies and were disproportionately represented in patients who experienced ill effects from their pre admission medication The high incidence of potentiation reflects the difficulty experienced in assessing the possible mechanisms of reactions by clinical means when the affected patient was subject to the simultaneous administration of several drugs with similar pharmacological properties

Drugs involved in adverse drug reactions (Table 8)

Although at least one adverse reaction occurred with eight of the ten most frequently prescribed drugs ampicillin caused more ill effects than any other single preparation In hospital however the anti neoplastic drugs

Table 7 *Mechanism of 119 adverse reactions*

Mechanism	Number of reactions	Reactions to drugs prescribed by general practitioners	Percentage of total reactions
Intended effect excessive			
With overdosage	6	2	5 0
Without overdosage	2	0	1 6
Potentiation	9	0	7 6
Pharmacological side effect			
With overdosage	7	2	5 9
Without overdosage	8	0	6 7
Potentiation	50	0	42 0
Allergic	19	7	15 9
Secondary effect	9	2	7 6
Irritation	5	0	4 2
Mechanism unknown	4	0	3 3
Total	119	13	100

Table 3 *Most frequently prescribed drugs*

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Phenobarbitone was the most commonly used drug (Table 3) but preparations with antibacterial activity constituted the largest pharmacological group comprising 469 (21 5%) of the prescriptions Anticonvulsant/sedatives were the next largest group and were followed in frequency by radiodiagnostic agents

The deployment of drugs for different purposes in differing situations explains the relative frequency of barbiturate usage as these

Table 4 *Pre admission drug usage (590 patients)*

Types of drugs	Number of drugs taken	% of total
Antibacterials	195	20 [illegible]
Analgesics (simple)	122	1[illegible] 7
Skin preparations	81	8 5
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Total	12 (12)	13 (4)	4 (4)	90 (39)

Number of patients involved in parenthesis

Table 9 *Relationship of frequency of adverse reactions to diagnosis*

Disease	No of reactions detected	Percentage of total reactions (%)	No of patients experiencing reactions	No of patients with same diagnosis	Percentage of patients experiencing adverse reactions (%)
Acute leukaemia	44	36 9	13	13	100
Other malignant disease	33	27 7	4	9	44
Bacterial meningitis	5	4 2	4	23	17 8
Epilepsy	4	3 3	4	42	9 5
Chronic pyelonephritis	3	2 4	2	3	66
Bacterial endocarditis	3	2 4	1	3	33
Acute pyelonephritis (with other disorders)	2	1 7	2	13	15 7
Diabetes	2	1 7	2	10	20
Rheumatic carditis	2	1 7	1	4	25
Kaposi s eruption	2	1 7	1	1	100
Convulsion/U R T I	2	1 7	2	33	6
Bronchitis	1	0 8	1	30	3 3
Scabies	1	0 8	1	7	14

reactions In two cases this was made possible by effective treatment of the clinical effects of the reactions but in 68 (56%) reactions affecting 19 children the continuation of the treatment of the basic disease was of such importance as to prevent its alteration regardless of the severity of the adverse effects experienced In 41 of these reactions some form of symptomatic or supportive therapy was required

Treatment was given for the clinical effects of 61 adverse reactions On only 5 occasions was a specific antidote available and in the majority of cases the treatment was symptomatic Supportive therapy to prevent deterioration of the child s general condition was required on 23 occasions Despite the severity of a further 5 adverse reactions no treatment could be given as no appropriate therapy was available

Fifty three (44 5%) reactions did not require any treatment

DISCUSSION

Intensive drug surveillance has proved to be of particular value for the epidemiological investigation of adverse drug reactions as it has enabled workers to adopt a more general approach to the problem of drug induced disease The system can be used to detect previously unrecognised adverse drug effects as well as to investigate their prevalence and aetiology (11) and the comprehensive nature of the data generated by the method has allowed comparisons to be made between the drug usage and adverse reaction patterns of different countries (12)

Similar studies of paediatric patients have shown that the adverse drug effects experienced by sick children differ from those of adults and that the differences are attributable to the distinctive drug usage patterns of childhood (2 13) This study while confirming these findings has also shown that variations in the prescribing practices of different paediatric centres may alter the frequency with which reactions occur

Our patients received fewer drugs (0 26/patient/day) than did their American counterparts whose level of drug usage (0 44/patient/day) was similar to that of adults (1) Also the pattern of drug use was different symptomatic treatment being commoner in American children who often received the types of drugs prescribed by general practitioners in the United Kingdom For example it is of particular interest to note the wide use of aspirin

Table 8 *Drugs most frequently involved in adverse reactions*

Drug	No of adverse reactions	No due to possible potentiation	No of patients	Patients receiving same drug	Per centage
Prescribed in hospital					
Methotrexate	15	15	7	9	77
6 Mercaptopurine	17	17	7	9	77
Prednisolone	10	9	7	27	26
Vincristine	21	18	6	8	75
Cytosine arabinoside	9	6	4	5	80
Adriamycin	17	17	4	4	100
Phenobarbitone	4	4	3	79	4
Iron edetate	2	0	2	27	7
Insulin	2	0	2	32	6
Ampicillin	2	0	2	51	4
Prescribed by practitioners					
Ampicillin	7	1	7	102	6
Penicillin V	2	1	2	42	5
Metoclopramide	2	0	2	11	28
Triclofos	1	0	1	4	25
Diphenhydramine	1	0	1	7	14

were most frequently involved in adverse drug reactions reflecting the modern aggressive approach to the treatment of malignant disease and the lack of specificity of this drug group The types of drugs which caused adverse reactions before admission were completely different, however, and emphasised the frequent use of antibiotics and symptomatic preparations in general practice The overall risk to patients of these drugs could not be assessed due to the lack of complete and accurate denominator data

Factors predisposing to adverse drug reactions

Adverse drug reactions occurred most frequently in children who suffered from serious diseases which presented a difficult therapeutic challenge (Table 9) Seventeen patients with malignant disease (2% of admissions) constituted 33% of affected patients and experienced 77 (64 7%) adverse reactions Similarly patients in other categories often suffered from complicated disorders such as epilepsy with proven brain abnormality or pyelonephritis complicating structural renal defects These therapeutic difficulties may also be reflected in the demonstrable relationship between increasing drug usage and the number of adverse drug reactions experienced As in other studies we found that the number of reactions increased significantly when children received more than four drugs during their stay in hospital ($p=0\ 01$) We were, however unable to demonstrate any predisposition to adverse drug reactions by age or sex

Severity of adverse drug reactions and effect on management

During the study no deaths were directly attributable to the adverse effects of drugs but 2 (1 7%) adverse reactions were considered life threatening Twenty four (20%) reactions were severe 56 (47%) were of moderate severity and 37 (31%) were mild

Adverse drug effects caused therapy to be discontinued on 12 occasions but on 8 of these effective alternative preparations were administered A further 18 reactions necessitated some reduction of the dosage of the offending drug or drug combination Nineteen (16%) reactions occurred after the completion of a course of therapy and 7 of these required treatment for the resulting clinical effects

Thus the basic treatment of the underlying condition remained unaltered after 70 (59%)

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Fifty three (44 5%) reactions did not require any treatment

DISCUSSION

Intensive drug surveillance has proved to be of particular value for the epidemiological investigation of adverse drug reactions as it has enabled workers to adopt a more general approach to the problem of drug induced disease The system can be used to detect previously unrecognised adverse drug effects as well as to investigate their prevalence and aetiology (11) and the comprehensive nature of the data generated by the method has allowed comparisons to be made between the drug usage and adverse reaction patterns of different countries (12)

Similar studies of paediatric patients have shown that the adverse drug effects experienced by sick children differ from those of adults and that the differences are attributable to the distinctive drug usage patterns of childhood (2 13) This study while confirming these findings has also shown that variations in the prescribing practices of different paediatric centres may alter the frequency with which reactions occur

Our patients received fewer drugs (0 26/patient/day) than did their American counterparts whose level of drug usage (0 44/patient/day) was similar to that of adults (1) Also the pattern of drug use was different symptomatic treatment being commoner in American children who often received the types of drugs prescribed by general practitioners in the United Kingdom For example it is of particular interest to note the wide use of aspirin

in the USA while this drug was prescribed for only four of our patients

These variations of prescribing practice may in part explain why we encountered fewer adverse drug reactions Other paediatric studies have reported adverse reaction rates of 10–13 % with 3 % of patient admissions caused by adverse drug effects while in our experience drug induced disease (adverse reactions plus accidental poisoning) was responsible for 4 6 % of admissions but there were fewer reactions to hospital therapy (6 5 %) Reported prevalence rates for adults vary widely from 25–30 % in the United States and New Zealand to 10 6 % in Great Britain (7 12 14)

Thus the incidence of drug induced disease arising from the community was similar in both countries but fewer of our patients were affected in hospital Comparison of the available data suggests that this may have been due to three principal factors

(*a*) our practice of withholding antibiotic therapy from children with presumed virus infections

(*b*) infrequent use of symptomatic preparations

(*c*) and differences of approach to the treatment of serious disorders e g meningitis which may have minimised the exposure of our patients to drugs with a high incidence of side effects e g ampicillin

Despite the potential value of such comparisons in the epidemiological study of iatrogenic disease the clinical effects of adverse reactions on the patient and his progress must be appreciated if rational steps are to be taken to reduce their incidence The adverse effects produced must therefore be considered in relation to the severity of the basic disease and the efficacy of the treatment available thus producing a form of therapeutic audit (22)

In this study the majority of adverse reactions to hospital treatment occurred in patients with serious disorders and their clinical effects regardless of severity were frequently outweighed by the potential advantage of the basic therapy The high incidence of reactions to anti neoplastic therapy seen in both countries (13) must, therefore be balanced against recent improvements in the prognosis for patients with malignant disease (24)

On the other hand little is known about the prevalence of iatrogenic disease in the community or the therapeutic setting in which it occurs though recent studies (15) suggest that it may be a significant problem We considered that many of the reactions which caused admission to hospital could have been prevented and drug monitoring in that area may indeed be profitable Continued surveillance of out patients would also be desirable in view of the high incidence of adverse drug effects experience by adults after discharge from hospital (10)

Community based studies could well show that comprehensive drug surveillance in hospitals highlights only a small proportion of drug induced disorders and does so in a setting where little effective action can be taken to reduce the problem

ACKNOWLEDGEMENTS

We are grateful to Professor J H Hutchison for advice and to Mrs S Sweenie for computer analyses We also thank the medical nursing secretarial and pharmacy staffs for their willing co-operation This study was supported by the Higher Medicine Fund of the erstwhile Western Regional Hospital Board

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Submitted Sept 3 1976
Accepted April 6 1977

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Acta Pædiatr Scand 66 777–781 1977

CASE REPORT

FAMILIAL DYSAUTONOMIA IN A NON JEWISH CHILD

HARALD ØRBECK and GUNNAR OFTEDAL

From the Department of Paediatrics Rikshospitalet University of Oslo Norway

ABSTRACT Ørbeck H and Oftedal G (Department of Paediatrics Rikshospitalet University of Oslo Norway) Familial dysautonomia in a non Jewish child Acta Paediatr Scand 66 777 1977 —Few documented cases of Riley Day syndrome fulfilling current diagnostic criteria have been recognized in non Jews In our case the diagnosis was established in a Norwegian child despite the absence of Jewish origin It represents a report of this syndrome with bilateral pathological changes in the hypothalamus in addition to extensive abnormal findings in the spinal cord and the autonomic ganglia These findings may have significance with regard to the pathogenesis of the disease

KEY WORDS Familial dysautonomia

This paper reports a Norwegian child of non Jewish ancestry with Riley Day syndrome Necropsy study revealed pathological lesions in the spinal cord and autonomic ganglia in addition to slight changes in the hypothalamus and in some of the hypothalamic nuclei Riley (11) suggested in his original description of the syndrome that familial dysautonomia is an autosomal recessive disease and later investigations have verified this view (5) The striking and bizarre symptomatology points at dysfunction of the sensory and the autonomic nervous systems and includes diminished lacrimation hyperhidrosis transient skin blotching Other features are indifference to pain and diminished deep tendon reflexes whereas impaired temperature control and frequent vomiting may imply a hypothalamic involvement The loss of histamine flare response and the absence of fungiform papillae on the tongue are consistent findings (16) Pharmacologic physiologic and pathologic anatomic investigations have more sharply defined the clinical entity (2 5 15) but the basic mechanism of the disease remains unknown

CASE HISTORY

The infant K A P born November 1968 was the first child of Norwegian parents Two siblings are healthy The infant was delivered 4 weeks preterm with a birthweight of ?480 g During the first year the child regurgitated constantly Walking was delayed beyond the second year of age the gait was awkward and atactic and for moving around he preferred to use a tricycle Many burn injuries were incurred Excessive night sweating and frequent vomiting were prominent features Prior to these episodes pronounced drivelling and nausea occurred with skin blotching Feeding was slow and accompanied by regular spluttering Recurrent bouts of pneumonia developed as well as a number of unexplained episodes of fever

From four years of age repetitive vomiting crises occurred which necessitated hospitalization for rehydration Clinical evaluation showed absent deep tendon reflexes hypolacrimation anisocoria and absence of corneal reflexes The child's sensation to pain and temperature on legs and arms was diminished but seemed intact on the abdomen Visual inspection of the tongue which was uniformly smooth revealed lack of fungiform papillae The histamine flare response was absent Ocular instillation of ? 5% methacholine induced *miosis* Most of his final year was spent in hospital with repeated episodes of vomiting and aspiration pneumonia *fluctuating* temperature and vasomotor instability Chlorpromazine treatment appeared to alleviate the child's apprehension and initially gave some *symptomatic* relief However subcutaneous injection of the parasympathomimetic agent Bethanechol and later the anticholinesterase drug Pyridostigmine bromide over prolonged periods did not



Fig 1 Diffuse astrogliosis in lateral hypothalamic area (arrows)



Fig 3 Atrophy of the posterior fasciculi in the spinal cord

reveal any beneficial effects In fact his condition worsened as his vomiting attacks became more severe In addition the boy was afflicted by recurrent episodes of pneumonia Despite postural drainage and administration of broad spectrum antibiotics he continued to develop respiratory distress and arterial hypoxia Assited ventilation eventually became necessary The child died in the terminal complication of heart failure and hypoxia at an age of eight years his weight was 20 kg and height 118 cm

Neuropathological examination

The brain weight was 1190 g which is at the lower range of normal The gross inspection of the brain revealed nothing abnormal except a thin corpus callosum and a slightly rounded shape of the posterior part of the 3rd ventricle

Microscopy from representative areas of the brain was performed The only irregular features were observed in the hypothalamus where subependymal glial granulations protruded from the posterior parts of the walls A diffuse astrogliosis was present around small vessels and also scattered in the lateral hypothalamic area and in the ventromedial thalamic nucleus (Fig 1) The nerve cells in the paraventricular (Fig 2) and supraoptic nuclei showed unusually large vacuoles in their cytoplasm Counting of cells in this area was not performed so the impression gained of a decrease in number of nerve cells in the area could not be supported

In the spinal cord a marked demyelination and atrophy was observed in the posterior fasciculi (Fig 3) The nerve cells in the column of Carke (Fig 4) appeared reduced in number and partly also atrophic The intermediolateral cell column appeared normal



Fig 2 Large vacuoles in nerve cells in the paraventricular nucleus



Fig 4 Unsymmetrical atrophy of the column of Clarke



Fig 5 Spinal ganglion showing disappearance of neurons and residual nodules of Nageotte (dark cell clusters)

The spinal ganglia were difficult to locate because of their small size The ganglion cells had completely dis appeared leaving residual nodules of Nageotte to indicate their location (Fig 5) Some lymphocytic infiltration was present and the nerve fibers were atrophic

Sections from muscles in the lower and upper extremi ties showed unspecific atrophy probably due to inactiv ity

Finally the lacrimal glands were small and firm with increased connective tissue and scattered as well as peri ascular lymphocytic infiltration

DISCUSSION

Occasional involvement of the autonomic nervous system has been described in many neurological conditions Among specific dys autonomias perhaps the best known is the Riley Day syndrome a congenital progressive familial dysautonomia in which clinical re covery does not occur At the age of five years our patient displayed all cardinal symptoms and signs so far described and fulfilled all the criteria listed by Riley & Moore (12) A section from the dorsum of the tongue failed to reveal any fungiform papillae The diagnosis was confirmed by a positive intradermal histamine test (14) The term familial dysautonomia was originally applied to Jewish children with an unusual symptom complex extensively de scribed by Axelrod et al (4) The disease ap pears to have propagated by an autosomal re cessive mode of inheritance through the cus tom of intermarriage among Jews Few in stances of familial dysautonomia in non Jews have been reported However in the present case both parents were of Norwegian descent A close investigation revealed that no mar riage with Jewish individuals had occurred Until 1851 Jews were not granted admission to the country of Norway

A disorder of catecholamine release has been proposed as a possible pathogenetic mechanism in the Riley Day syndrome In general an elevated urinary HVA/VMA ratio has been reported (18) and in 25% of the cases a low level of serum dopamine β hy droxylase activity has been found (20) On the other hand one recent survey (7) reports no difference in serum dopamine β hydroxylase activity between 34 familial dysautonomia patients and a control population None of the above chemical findings were made in this case in spite of repeated tests Evidently such assays are not indispensable for the diagnosis of familial dysautonomia

The basic defect in familial dysautonomia is currently obscure Among previous explana tions nerve dysfunction involving acetyl choline activity has been put forward since parasympathetic insufficiency is the most striking clinical finding This would imply a defect in either acetylcholine synthesis or in the mechanism for its storage or release (9) The possibility of a receptor malfunction also arises and Drachman (6) suggests that acetyl choline may somehow mediate the trophic in fluence of the nerve It has been argued that a decreased number of unmyelinated fibres in peripheral nerves reported in several in stances (2 9) implies that peripheral nerve dysgenesis may be the underlying cause Siggers et al (13) found elevation of nerve growth factor in serum from patients with familial dysautonomia and proposed a poten



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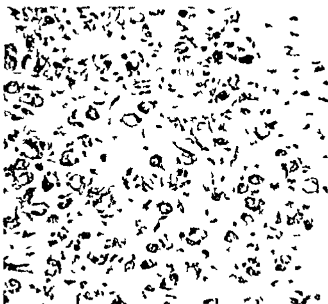


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Fig 4 Unsymmetrical atrophy of the column of Clarke at the upper thoracic level



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Submitted Febr 17 1977
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Though the diverse manifestations of this disease seem to suggest a central cause no consistent brain abnormality has been reported Sparsity of myelinated fibres in the dorsal roots and a symmetric degeneration of fasciculus interfascicularis in the dorsal spinal tract appear to emerge as the most characteristic neuropathologic manifestations of the disease (19)

In our case the brain weight was at the lower normal level and corpus callosum appeared thin The subependymal granulations in the walls of the posterior hypothalamus indicated some slight damage to the ependymal cells and the slight gliosis observed in the lateral parts of the hypothalamus and in the ventromedial thalamic nucleus pointed in the same direction The striking vacuolation of the nerve cells in the paraventricular and supraoptic nuclei may be regarded as an indication of hyperactivity in these cells The scattered observations made in the brain—although limited to the hypothalamic area—do not give conclusive clue regarding the nature of the basic disturbance in this disease

The initial report of the syndrome by Engel & Aring (3) presented before the syndrome had been named showed somewhat similar abnormalities Their finding of a thalamic cyst may not have been a coincidental lesion but a result of degenerative alterations on the basis of the same changes found in our patient The central manifestation noted in this case and the inconsistency of pathological findings described may be due to some metabolic change which also could be the answer to the cyclic nature of the disease

Although recent reports indicate improvements in survival life remains a struggle for these children and their families It should be noted however that despite the rarity of the disease a parent organization is founded in the United States in an effort to find help and hope for these handicapped children

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Acta Pædiatr Scand 66 783–785 1977

CASE REPORT

MONOZYGOTIC TWINS CONCORDANT FOR TRACHEO ESOPHAGEAL FISTULA AND DISCORDANT FOR THE VATER ASSOCIATION

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From the Departments of Pediatrics and Surgery the Pennsylvania State University College of Medicine Hershey Pennsylvania USA

ABSTRACT King S L Ladda R L and Shochat S J (Departments of Pediatrics and Surgery Pennsylvania State University College of Medicine Hershey Pennsylvania USA) Monozygotic twins concordant for tracheo-esophageal fistula and discordant for the VATER association Acta Paediatr Scand 66 783 1977 —Monozygotic female twins were concordant for tracheo-esophageal fistula (TEF) and one twin also manifested other anomalies of the VATER association including agenesis of the female reproductive tract Review of the literature discloses a familial tendency for recurrence of TEF and a high concordance rate in monozygotic twins indicating a significant genetic influence for the isolated anomaly In the case of the VATER association the sporadic occurrence of affected individuals and discordance in twins implicates the effect of non genetic factors

KEY WORDS Tracheo-esophageal fistula VATER association *monozygotic twins female genitalia*

Tracheo-esophageal fistula (TEF) may occur as an isolated anomaly or associated with one or more defects Quan & Smith (14) noted that certain of these anomalies occurred together more frequently than expected by chance alone and they proposed the acronym VATER to denote the apparently non random association of vertebral anomalies anal atresia TEF and renal and/or radial dysplasia More recently malformations of the male external genitalia have been described as part of this association of anomalies (1) We wish to report a case of monozygotic female twins who were concordant for tracheo esophageal fistula One twin also exhibited anomalies characteristic of the VATER association as well as agenesis of the female reproductive tract A similar combination of malformations in twins has not been reported previously

CASE REPORT

Monozygotic female twins were born at approximately 32 weeks gestation to a mother in good health Zygosity of the twins was established by examination of the placenta it had a single chorion and a single amnion The pregnancy was complicated by polyhydramnios Both twins were cyanotic at birth and required intubation and positive pressure O_2 Several hours after birth the twins were found to have TEF and they were transferred to our medical center for care

Twin A weighed 1360 g at birth physical examination revealed no abnormalities An X ray study revealed a blind upper esophageal pouch consistent with the diagnosis of TEF with esophageal atresia (Type C) Skeletal X rays intravenous pyelogram (IVP) and electrocardiogram (ECG) revealed no further abnormalities

Twin B weighed 1 00 g and was noted to have an imperforate anus with a fistula to the vulvar area and no vaginal orifice X ray study also revealed a blind esophageal pouch confirming the diagnosis of TEF with

Special Trainee in Medical Genetics on leave from Northwestern University School of Medicine Fourth Year Class

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Submitted March 7 1977
Accepted April 21 1977

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esophageal atresia (Type C) Skeletal X rays disclosed two left hemi vertebrae and fusion of two pairs of ribs in the right thorax IVP showed an ectopic midline pelvic kidney with a single ureter ECG was normal This infant died after three months and autopsy revealed agenesis of the fallopian tubes uterus and vagina The karyotype was normal

DISCUSSION

Six sets of twins concordant for TEF have been reported (4 5 7 11 19) In five twins in which zygosity was determined four were monozygotic and one set was dizygotic Although isolated TEF generally has been a sporadic occurrence in an otherwise normal family it has recurred in families most often in the offspring of normal parents (8 10 17) Engel et al (10) described TEF in a mother and her child and Schimke et al (17) found five members of one family affected In contrast individuals considered to have the VATER association (3 or more defects) have all occurred sporadically (1 14 18) In one family TEF and imperforate anus occurred in three siblings of normal parents (12) but no other defects were noted In only one other instance has the VATER association occurred in twins and as in our case only one twin was affected (18) Thus TEF is associated with a definite familial tendency whereas the VATER association is not

Barry & Auldist (2) reported that 43% of their patients with TEF had at least one other major abnormality the most frequently occurring being those of the VATER set of anomalies They suggested that the VATER association should be considered as part of a spectrum that ranges from the occurrence of one defect alone such as TEF to the full set of VATER anomalies However instances of the VATER association have all been sporadic and with the exception of our monozygotic twins, we are unaware of the occurrence of isolated TEF and the VATER association in near relatives On the other hand isolated TEF is associated with a familial tendency This suggests that the etiology of isolated TEF may differ from that of TEF occurring with the VATER association

Isolated TEF is estimated to occur from 1 in 800 to 1 in 2 500 live births (3 13) The familial tendency for TEF (simulating autosomal recessive and dominant inheritance patterns) and the increased occurrence in near relatives (monozygotic twins siblings and offspring) compared to the general population suggests a multifactorial mode of heritability (6) Applying Edwards (9) empiric formula ($\sqrt{\text{population incidence}}$) an estimate of the recurrence risk of TEF in first degree relatives may range from 2–3 5% i e 25–50 × greater risk than the general population Because of this increased recurrence risk with the economic and psychological burden associated with the surgical correction and long range treatment of TEF prenatal diagnosis (amniography) may be offered in subsequent pregnancies Contrast material injected into the amniotic fluid is swallowed by the fetus outlining the gastrointestinal tract and permitting the diagnosis of TEF (15)

The occurrence of genital anomalies with the VATER association is a recent observation (1 18) Genital malformations noted by Apold et al (1) in 3 of 7 male patients with the VATER association included bifid scrotum dysplastic penis and hypospadius with testicular atrophy They also called attention to earlier reports of hypospadius and female pseudohermaphroditism in other patients with the VATER set of anomalies (16 18) In twin B the female reproductive system was completely absent not even the external vaginal orifice was present The severity of this defect in our patient suggests a total failure of Mullerian differentiation

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Acta Pædiatr Scand 66 787–788 1977

CASE REPORT

TRISOMY 20 MOSAICISM

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ABSTRACT Carbonell X Caballin M R Rubio A and Egozcue J (Department of Neonatology Instituto Corachan and Instituto de Biologia Fundamental Universidad Autonoma de Barcelona Barcelona Spain) Trisomy 20 mosaicism Acta Paediatr Scand 66 787 1977 —The first known case of trisomy 20 mosaicism is described As in other cases of (partial) trisomy 20 the patient showed scarce physical malformations It is suggested that trisomies for chromosomes of the F group are rare not because they are lethal but as a result of the morphology of the chromosomes involved

KEY WORDS Trisomy mosaicism chromosome 20

According to Sandler & Hecht (5) trisomy 19–20 is rare in spontaneous abortions and unknown in live births In the pre banding era several cases of partial F trisomy were published (1 4) but in all of them the chromosomes were identified by morphology alone Carrel et al (2) using autoradiography identified one case of partial F trisomy resulting from a familial F/13 translocation More recently Šubrt & Brychnač (6) described one case of trisomy for the short arms of chromosome 20 using banding techniques this case of partial trisomy was due to a 20/21 translocation

In this paper we report a case of regular trisomy 20 mosaicism identified by G banding techniques

CASE REPORT

The patient was an 8 month-old Caucasian male born to unrelated parents He was seen by the pediatrician due to a persistent anorexia The physical examination revealed a well developed pale obese child with a slight turricephala mongoloid slant of the eyes and internal epicanthus A simian crease was present in the right hand All other studies performed were normal

CYTOGENETIC STUDIES

The examination of Giemsa stained slides from routine blood cultures revealed the presence of two cell lines one with 46 chromosomes (60%) and the other with 47 chromosomes (40%) The extra chromosome seemed to belong to the F group

G bands were obtained using a technique described by us (3) According to its banding pattern the extra chromosome was identified as a no 20 (Fig 1)

DISCUSSION

To our knowledge this is the first confirmed case of regular trisomy 20 mosaicism The main interest of the case resides in the fact that none of the two cases confirmed by banding techniques (one partial one mosaic Šubrt & Brychnac (6) and present case) resulted in severe malformations In the case of Šubrt & Brychnáč the child had a mild mental subnormality epicanthus arched palate maxillary hypoplasia spondylodysplasia thoracic kyphosis and lumbar lordosis In our case the signs were so minimal that neither the obstetrician nor the parents had paid any attention to them This suggests that trisomy 20

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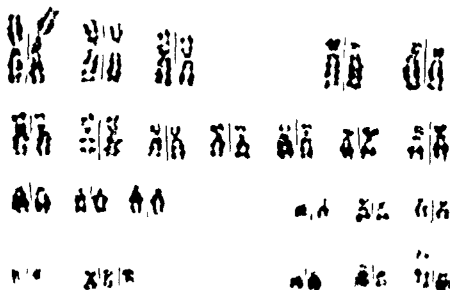


Fig 1 G banded karyotype showing trisomy 20

may not have an extreme deleterious effect as was previously thought It is possible that trisomies of the F group are scarce not because they are nonviable but due to the fact that the morphology of the chromosomes (metacentric) by regularly producing two chiasmata during meiosis I prevents their precocious separation and non disjunction

REFERENCES

1 Bartalos M & Baramki T A *Medical cytogenetics* The Williams & Wilkins Co Baltimore Md 1970 p 336
2 Carrel R F Sparkes R S & Wright S W Partial F trisomy associated with familial F/13 translocation detected and identified by parental chromosome studies *J Pediatr 78* 664 1971
3 Egozcue J Caballin M R & Godav C Banding patterns of the chromosomes of man and the chimpanzee *Humangenetik 18* 77 1973
4 Hamerton J L *Human cytogenetics* Academic Press New York N Y 1971 vol 2 p 365
5 Sandler L & Hecht F Genetic effect of aneuploidy *Am J Hum Genet 25* 332 1973
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Submitted Febr 14 1977
Accepted March 29 1977

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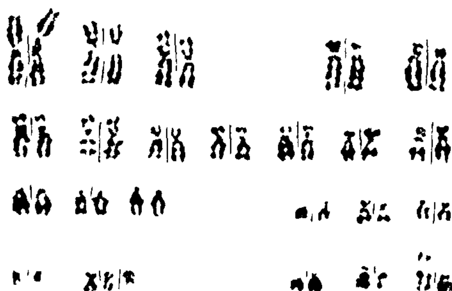


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of the different postural reflexes The translation is made by Professor H H Matthiass Dr M Feldkamp and Dr A Boroske and is clear and easy to read

I would like to recommend this book for those who are interested in cerebral palsy and have not read it before It gives the reader a comprehensive idea of cerebral in fluen e in movement and how it can be used in examina tion and therapy according to the Bobath method

Ingrid Bjerre

J C Somogyi & Tashev (eds) *Early signs of nutritional deficiencies* 173 pp illus Proceedings of the 13th Sym posium of the Group of European Nutritionists Sofia and Varna May 8-31 1974 Bibliotheca Nutritio et Dieta No 23 S Karger Basel 1976 sFr/DM 98 — ISBN 3 8055 2314 9

The lectures from the 13th Symposium of the Group of European Nutritionists in 1974 are now available in book form To detect slight deficiencies in the metabolism of protein EFA vitamins minerals or trace elements methods are needed for an early diagnosis A revue of early clinical and biochemical signs of protein calorie malnutrition (PCM) is given in the book It is now pos sib e to select those children or those groups of children who are at risk of developing PCM

Malnutrition following gastric resection depends in part on morphologic and especially on enzymatic deficiencies of the intestinal mucosa Lactase and other disaccharidase deficiencies underlie the impaired digestion and absorp tion of milk and carbohydrates Clinical and chemical diagnostic signs of polyunsaturated fatty acid deficiency are given An impaired platelet function and other hema t logi al disturbances in essential fatty acid deficiency are of great interest Food deficiency diseases and the en zymatic adaptation to protein deficiency are presented and termed diseases of imbalances of nutrition at the cellular level

Good analytical methods are now available for measur ing vitamin A E thiamine (transketolase activity) and the niacin metabolite (N methyl nicotinamide) The advan tages and disadvantages of different radioisotopic meth ods in the diagnosis of vitamin B ₁ are discussed The lec tures con erning iron deficiency are of minor interest The commonly used parameters have een applied on dif ferent pc pulation groups Evaluati n of the methods used i lacking and no l nes for the future are given Aspects of e g ferritin-determinat on iron isotop techniques and wh le body counting are mi sing

In the chapter f cal ium and phosphorus deficiency the tatements by FAO/WHO in 1962 are referred to no lear-cut disea e due to cal ium deficiency has ever been described At pre ent no single biochemical test can be used f r the early diagnosis of trace-element (Zn Cr Cu) defi ency in man The most promising line will be the ssay of mi r element-dependent enzymes

In on lus n the boo k is of interest for all working with nutriti nal pr blems The results of the symposium h d been more useful if the editors had included the dis u ns and if the chairmen had summarized each se tion

Gosta Samuelson

F A Hommes & C J van den Berg (eds) *Normal and pathological development of energy metabolism* 246 pp illus Academic Press London New York San Francisco 1975 £7 50

This book is the result of a meeting on the relation be tween developmental biochemistry and inborn errors of metabolism associated with brain damage held in the Netherlands in October 1974 The volume contains 18 presentations held by invited specialists in pediatrics biochemistry and genetics The success of this inter disciplinary approach is evident from the included stimulating discussion following each presentation The papers are uniformly of a high quality and summarize recent research data of basic character that could not easily be collected from their sources

Introductory papers deal with energetic aspects of late fetal and neonatal metabolism factors influencing growth and differentiation of the embryonic rat pancreas hor monal regulation of enzyme synthesis and the influence of food on body composition of low birth weight infants Developmental changes in carbohydrate metabolism that occur in the rat liver are discussed in two articles and evidence is presented showing that the rate of gluconeo genesis from lactate is higher in the infant as compared to the adult animal whereas the contribution of the alanine cycle to gluconeogenesis is low during the neonatal period Another interesting contribution describes the effect of ammonia on metabolic processes in isolated liver cells addition of ammonium chloride increased the energy demand of the cells and it was suggested that this observation could contribute to the understanding of ammonia toxicity Other papers deal with biochemical changes in diseases caused by inborn errors of metabo lism such as galactosemia fructose intolerance phenyl ketonuria Maple syrup disease and a more recently described condition with deficiency of hepatic methionine adenosyltransferase

A series of papers concern substrate utilization in the developing brain The high utilization of ketone bodies by the brain during growth is discussed by different authors including Land and Clark who suggest that ketone bodies are important as substrate for both synthesis and energy production in the developing brain In a succeeding paper Clark and Land also present data which indicate that the transport of both pyruvate and 3 hydroxybutyrate into the brain mitochondria can be inhibited by phenylpyruvate and 2 oxo-4 methyl pentanoate Based on these observations the authors suggest that an inhibited transport of pyruvate and 3 hydroxybutyrate into the mitochondria could in part ex plain the defective myelination and depressed energy metabolism seen in phenylketonuria and Maple syrup disease Finally Blass and co-workers have written a very helpful review on the clinical and metabolic abnormalities which accompany deficiencies in pyruvate oxidation

This book covers a wide range of topics It might there fore have been helpful if the papers had included a brief summary and if the different articles had been arranged under different headings The book should be of interest for pediatricians with special interest in inborn errors of metabolism and developmental brain biochemistry

Gisela Dahlquist *Bengt Persson*

BOOK REVIEWS

J F Bosma & J Showacre (eds) *Symposium on development of upper respiratory anatomy and function Implication for sudden infant death syndrome* U S Department of Health Education and Welfare Publication No (NIH) 75 941 Washington D C 1975 279 pp illus $7 10

Sudden unexpected infant death (SID) continues to be a challenge to medical research The incidence appears too low to permit rational longitudinal population studies with the purpose of early detection of factors to identify infants at risk but is far too high to be regarded as just a sad curiosity Although the number of proposed causes is large and increasing much interest has lately been focused on the possible role of reflexes elicited from the upper airways The proceedings of this symposium held in 1974 are consequently of great interest The list of distinguished participants reveals the aim of the organizers to stimulate an interdisciplinary discussion at a high level

An interesting introductory chapter deals with the anatomy of the upper respiratory tract in the newborn in relation to its positional respiratory and feeding functions and to their postnatal development Illustrations are numerous and informative Aspects of the embryology of the region are presented with special attention given to the neural crest in a following chapter Large space is devoted to a paper on the mechanics of the larynx in adults but due to well known anatomical differences its relevance to infants is doubtful The distribution of blood and lymph vessels in the region is demonstrated with interesting lymph vessel connections between the palate and the pharynx The morphological section is ended by a well illustrated paper on the development of sensory receptors in oral mucosa Morphology has hitherto given few clues to the riddle of SID One serious problem is that present knowledge of normal variations in upper respiratory tract anatomy in infants is far from complete

The development of the control of respiration is a wide and rapidly progressing field An up to date review would have been more appropriate than the paper presented which partly deals with lung mechanics and includes some puzzling information The reference list ends at 1967

Some excellent papers are devoted to the induction of apnea in animals by stimulation of sensory receptors in the upper airways and in the larynx Recent work has revealed receptors which respond to water and to milk from other species This may represent a breakthrough in SID research as animal models are now available to study a physiological process which may be significant in human SID The possibility that SID victims are hyporesponders to respiratory stimuli is also discussed in an interesting paper and methods for testing this are presented in another one

The value of the book is further increased by reports from sometimes animated discussions of the papers and extensive indices of authors and subjects

Ola Hjalmarson

D A Fisher & G N Burrow (eds) *Perinatal thyroid physiology and disease* Kroc Foundation Series No 3 Raven Press Publishers New York 1975 277 pp illus US $20 95

The recent availability of highly sensitive and specific radioimmunoassay systems for measurement of thyroid hormones and TSH in biologic fluids has dramatically changed our understanding of thyroid physiology and disease Screening of newborn infants for congenital hypothyroidism has also become possible Thyroid hormone deficiency may at critical periods of human development severely limit central nervous system maturation To date it is not known with certainty whether early treatment will prevent mental retardation Because congenital hypothyroidism may occur as frequently as 1 in 5000 births the answer to this question is of extreme importance to the patient as well as to the parents and to society

This book gives an excellent review of present knowledge regarding these problems The first part concerns with normal thyroid physiology in both the mother and the foetus The second part deals with experimental studies on thyroid hormone in development with special reference to the central nervous system The third part concerns with thyroid disease in pregnancy and the newborn including treatment of and prognosis for children with congenital hypothyroidism treatment of thyrotoxicosis during pregnancy and a review of congenital Grave s disease In the last part of the book several contributors report on their experiences of newborn screening for hypothyroidism using various methods at various times during the neonatal period The results constitute a valuable base for future screening programs for congenital hypothyroidism

Karl Olof Nilsson

Berta Bobath *Abnorme Haltungsreflexe bei Gehirnschaden* 3rd ed 94 pp illus Georg Thieme Verlag Stuttgart 1976 DM 9 80 *ISBN 3 13-4351-03 X*

This book is a new edition of the German translation of the now classic work of Berta Bobath on abnormal postnatal reflex activity It was first published in English already in 1954

There is a short introduction by Mrs Bobath and the small volume is well illustrated with instructive pictures

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Alternative approaches to meeting basic health needs in developing countries V Djukanovic & E P Mach (eds) World Health Organization Geneva 1975 116 pp sFr 24 – ISBN 92 4 156047 7

This volume is the report of a study carried out jointly by UNICEF and WHO in 1973 and 1974 Faced with the failure of conventional health services to make an impact on the health problems of developing countries the two organizations decided to examine some of the successful or promising primary health care systems now operating in these countries in an attempt to pick out the factors responsible for their success

Part I of the report describes the underprivileged sections of the population who are often without even rudimentary health services While remedies are available for many of the shortcomings they often cannot be applied without changing the whole concept of health care

Part II presents descriptions of 10 successful or promising innovative health care programmes in different parts of the world There are programmes adopted nationally and those covering more limited areas

In part III of the report the editors draw a series of conclusions Despite the immense problems it is possible using the resources available to meet some of the people s basic health needs In the national programmes one of the crucial features has been a strong political will for change In the more local schemes enterprise and leadership have been vital

On the basis of the report WHO and UNICEF have taken decisions implying major changes in their own policies

The booklet is a fascinating reading for anyone concerned with health needs priorities and policies The examples of achievements reached by human involvement and leadership are far greater than most of us would ever dream of

The issues concern us much more than appears from the title How should self reliance in health be interpreted in various cultural and economic situations? How does one get community motivation and continuous participation in solving the health needs of any society? How should the patients right to democratic influence in the health care process be balanced against political democracy?

The studies presented in this volume could be regarded as a take off point for a new global strategy on health

Goran Sterky

J M Tanner R H Whitehouse W A Marshall J R Healy & H Goldstein *Assessment of skeletal maturity and prediction of adult height (TW2 Method)* pp illus Academic Press Inc London New York San Francisco 1975 £9 00

In 1962 Tanner Whitehouse & Healy published the method for assessment of skeletal maturity (TW1) Each bone of the hand and wrist was given a score depending on its development The sum of these scores formed an expression for the skeletal maturity This publication presents a revised version of the original method The previously used scoring system is retained essentially unchanged However the different scoring points have been recalculated according to a more valid mathematical and statistical model

The system furthermore provides separate maturities for 1) the carpal bones 2) the distal epiphysis of radius and ulna the metacarpals and phalanges (RUS) and 3) all the bones of the hand and wrist The RUS maturity is most valid in the prediction of adult height which is one of the important applications extensively discussed by the authors Another one is the correlation between the effect of different hormones and skeletal maturity

In everyday clinical work the TW2 method is as time consuming as the TW1 method was The method is of importance especially in assessment of skeletal maturity in patients with irregular development of the different bones in the hand and wrist

The prediction of adult height according to the present method has theoretical advantages as it takes into consideration 1) added residual standard deviation and 2) parental heights In practical situations the method gives values comparable to those of other methods As a research tool the TW2 method is ideal and the book ought to be available in every department where skeletal maturity is routinely assessed

Hans Ringert

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ABSTRACT Köhler L. and Holst K. (Department of Paediatrics, University Hospital, Lund Sweden) Dental health of four year-old children Acta Pædiatr Scand 00

An unselected population of 1 567 four year-old children

KEY WORDS Pre-school children caries gingivitis

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1 Brecher G & Stohlman F., Jr Humoral factors in erythropoietin. *In* L. M Tocantins & R. Penn (eds.) *Progress in hematology* Grune & Stratton, New York *1959 p 110*
2. Smith, C. A. *The physiology of the newborn infant* Thomas, Springfield, Ill 1967 3rd ed. vol 2, p 120
3 Vedra, B & Ulrych, J. Anaerobiosis in normal and asphyxiated premature newborns. 2. Four approaches. *Acta Paediatr Scand 49* 129 1960

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VOL 66 / SEPT 1977 / NO 5

ACTA PÆDIATRICA SCANDINAVICA

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SERUM IMMUNOGLOBULIN LEVELS IN THE COURSE OF ANAPHYLACTOID PURPURA IN CHILDREN

S SIMILA K KOUVALAINEN and M LANNING

From the Department of Paediatrics University of Oulu Oulu Finland

ABSTRACT Simila S Kouvalainen K and Lanning M (Dept of Paediatrics University of Oulu Oulu Finland) Serum immunoglobulin levels in the course of anaphylactoid purpura in children Acta Paediatr Scand 66 537 1977.—Serum levels of immunoglobulins (IgG IgA IgM IgD and IgE) were determined at frequent intervals in the course of anaphylactoid purpura (AP) in children AP was cured without complications in 16 out of 26 cases recurring in 7 cases Melena was manifested in 9 cases and nephropathy in 5 The levels of IgA and IgM were elevated in AP but serum IgG IgD and IgE showed no significant changes The serum level of IgM was significantly ($p<0.0125$) higher in patients with AP nephropathy than in those with recent AP The elevated serum IgA and IgM levels are possibly related to the pathogenesis of AP and/or its renal involvement

KEY WORDS Anaphylactoid purpura immunoglobulins nephropathy

The cause of anaphylactoid purpura (AP) an allergic vasculitis of childhood manifested by a characteristic skin rash and often also associated with arthritis gastrointestinal symptoms such as pain and melena or glomerulonephritis has remained unknown Histologically the skin (1) and renal lesions (10 19) in AP resemble those in certain immune complex diseases Selective elevation of serum IgA has been reported in most patients with AP (8 15 16)

In order to check the immunological response in children with anaphylactoid purpura serum immunoglobulin levels were determined several times in the course of the disease and the elevation of the serum IgA and IgM levels followed High IgM was associated with AP nephropathy

MATERIAL AND METHODS

St d up

Th dy group cons sted of 6 children 11 female and 1 with anaphylactoid purpura treated in the Department of Paediatrics University of Oulu during the 3 year period 1973–75 The basic criterion for including a patient in the study group was purpuric rash characteristically affecting mainly limbs and buttocks Other diseases with similar manifestations were excluded A history of infection occurring during the 4 weeks preceding AP was present in 77% of the children and evidence of streptococcal aetiology (positive culture elevated AST titre) was recorded in 5 patients Two of the patients had a history of allergy (atopic excema allergic rhinitis or bronchial asthma)

In the course of the disease serial determinations of serum immunoglobulins IgG IgA IgM IgE and IgD were performed varying in number between 2 and 6 The patients were divided into four subgroups according to the outcome of AP as follows (Fig 1)

1 patients with simple AP without any complications (all cured at the first attempt)
2 patients with AP and melena
3 patients with recurrent AP
4 patients with AP and nephropathy

Methods

The serum levels of IgG IgA IgM and IgD were determined by the single radial immunodiffusion method of Mancini (12) using commercially available antisera from Behringwerke The concentration of IgE was determined by a radioimmunosorbent technique ((9) commercial antiIgE antiserum from Pharmacia Uppsala) The accuracy of the methods was about ±10%

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SERUM IMMUNOGLOBULIN LEVELS IN THE COURSE OF ANAPHYLACTOID PURPURA IN CHILDREN

S SIMILA K KOUVALAINEN and M LANNING

From the Department of Paediatrics University of Oulu Oulu Finland

ABSTRACT Simila S Kouvalainen K and Lanning M (Dept of Paediatrics University of Oulu Oulu Finland) Serum immunoglobulin levels in the course of anaphylactoid purpura in children Acta Paediatr Scand 66 537 1977 —Serum levels of immunoglobulins (IgG IgA IgM IgD and IgE) were determined at frequent intervals in the course of anaphylactoid purpura (AP) in children AP was cured without complications in 16 out of 26 cases recurring in 7 cases Melena was manifested in 9 cases and nephropathy in 5 The levels of IgA and IgM were elevated in AP but serum IgG IgD and IgE showed no significant changes The serum level of IgM was significantly ($p<0\ 0125$) higher in patients with AP nephropathy than in those with recent AP The elevated serum IgA and IgM levels are possibly related to the pathogenesis of AP and/or its renal involvement

KEY WORDS Anaphylactoid purpura immunoglobulins nephropathy

The cause of anaphylactoid purpura (AP) an allergic vasculitis of childhood manifested by a characteristic skin rash and often also associated with arthritis gastrointestinal symptoms such as pain and melena or glomerulonephritis has remained unknown Histologically the skin (1) and renal lesions (10 19) in AP resemble those in certain immune complex diseases Selective elevation of serum IgA has been reported in most patients with AP (8 15 16)

In order to check the immunological response in children with anaphylactoid purpura serum immunoglobulin levels were determined several times in the course of the disease and the elevation of the serum IgA and IgM levels followed High IgM was associated with AP nephropathy

MATERIAL AND METHODS

Study group

The study group consisted of 6 children 11 female and 15 male with anaphylactoid purpura treated in the Department of Paediatrics University of Oulu during the 3 year period 1973–75 The basic criterion for including a patient in the study group was purpuric rash characteristically affecting mainly limbs and buttocks Other diseases with similar manifestations were excluded A history of infection occurring during the 4 weeks preceding AP was present in 77% of the children and evidence of streptococcal aetiology (positive culture elevated AST titre) was recorded in 5 patients Two of the patients had a history of allergy (atopic excema allergic rhinitis or bronchial asthma)

In the course of the disease serial determinations of serum immunoglobulins IgG IgA IgM IgE and IgD were performed varying in number between 2 and 6 The patients were divided into four subgroups according to the outcome of AP as follows (Fig 1)

1 patients with simple AP without any complications (all cured at the first attempt)
2 patients with AP and melena
3 patients with recurrent AP
4 patients with AP and nephropathy

Methods

The serum levels of IgG IgA IgM and IgD were determined by the single radial immunodiffusion method of Mancini (1) using commercially available antisera from Behringwerke The concentration of IgE was determined by a radioimmunosorbent technique ((9) commercial anti IgE antiserum from Pharmacia Uppsala) The accuracy of the methods was about ±10%

ACTA PÆDIATRICA SCANDINAVICA

Subscription and Distribution
The Almqvist & Wiksell Periodical Company
26 Gamla Brogatan
S 101 20 Stockholm 1, Sweden

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ent results that this elevation is independent of the severity of AP or any relapses or com plications occurring After long term recovery the high IgA level will be normalised (8) The cause of the elevated IgA is unknown It has been suggested that a local IgA antibody re sponse in the gastrointestinal tract might oc cur in AP and result in the increased serum IgA globulin levels (16) as Immonen (7) and Savilahti (14) have nicely demonstrated to oc cur in celiac disease It has been demonstrated in skin and rectal biopsies from patients with AP that vessel walls and dermo-epidermal junctions are particularly prone to contain de posits of IgA complement C3 and fibrinogen but there may also be some containing IgG and IgM (2 17)

The present results also indicate that the level of serum IgM is elevated in patients with recent AP and that it is obviously high in patients with AP nephropathy (Table 1) The main types of glomerular lesions in AP neph ropathy are minimal lesions diffuse endocapil lary proliferation focal and segmental glo merulonephritis membranoproliferative glo merulonephritis and endo- and extracapillary glomerulonephritis Deposits of immunoglobu lins mainly IgA C3 and fibrinogen but also IgM and IgG have been observed within the mesangium and also along the glomerular cap illary walls (13) In this respect AP nephro pathy resembles idiopathic mesangial IgA glo merulonephritis or Berger s disease (4) Pa tients with Berger s disease have elevated serum IgA levels but normal serum levels of IgM and IgG (11) It is suggested that in Berger s disease C3 is most probably activated by the alternate pathway possibly by aggre gates of IgA It is possible that the same al ternative pathway is also activated in AP by aggregates of IgA and IgM (5) It has also been noted in experimental animals that humoral antibodies against certain glomerular antigens (nephrotoxic antibodies) may reside in IgM (18) The association is probably also patho-genetically noteworthy in this respect In fact the histologic pictures of nephrotoxic serum nephritis and AP nephropathy possess a cer tain mutual resemblance (6 19) As far as we know however no clear cut proof has been presented for the existence of antiglomerular antibodies in AP On the other hand infec tion precedes AP in most cases (77% in this series) and may also have occurred in the re maining patients in a latent form Micro-orga nisms may differ in respect of the antibody response they cause certain microbes especi ally those with polysaccharide surface anti gens stimulating antibody response of the IgM type As giant molecules IgM antigen complexes would most probably be tagged in the glomerular capillaries causing damage there by complement activation

Could it be that AP with nephropathy may arise in patients who have experienced an in fection with pronounced antibody response of the IgM type? All 5 patients with elevated IgM and nephropathy in the present series had had preceding infections

Whatever the cause and pathogenic signifi cance of the elevated IgM levels in AP we emphasize that it may serve as an outstanding sign of more severe disease and a prediction of a poorer outcome

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Table 1 *Serum levels of five immunoglobulins (mean ±S D) in patients with anaphylactoid purpura (AP)*

Number of patients is shown in parentheses

Clinical manifestations	IgG (g/l)	IgA (g/l)	IgM (g/l)	IgE (U/l)	IgD (mg/l)
A Initial stage *recent AP*	*(26) 10 93±3 56*	*(26) 2 60±1 26*	*(26) 1 34±0 73*	*(22) 129±273*	*(18) 36±47*
B Later stages					
1 Healed AP without complications	(10) 10 89±3 94	(10) 2 71±0 98	(10) 1 22±0 72	(10) 187±363	(9) 44±59
2 AP with melena	(9) 9 54±3 11	(9) 2 42±0 64	(9) 1 84±1 07	(9) 297±634	(7) 79±76
3 Recurrent AP	(7) 11 26±3 87	(7) 2 90±1 54	(7) 1 93±0 91	(7) 272±403	(6) 38±72
4 AP with nephropathy	(5) 9 30±4 07	(5) 3 07±1 12	(5) 2 81±1 36	(5) 93± 55	(5) 46±76
Normal values					
Immonen 1968	*10 18±3 41*	*0 64±0 49*	*0 66±0 38*		
Berg & Johansson 1969				105± 70	26±18

$p<0\ 0125$ as compared with group A

RESULTS

The concentrations of IgG IgA IgM IgD and IgE in the total series are shown in Table 1

The level of IgG was similar to that in healthy Finnish children of the same ages (7) and no significant differences were noted in the serum IgG levels of the four AP subgroups

The concentration of IgA was significantly elevated ($p<0\ 001$) in the patients with recent AP and remained at the same level in those with recurrent purpura melena or haematuria At 3–16 (mean 8) days after the healing of an uncomplicated anaphylactoid purpura the serum IgA was still at the same high level

The level of IgM in patients with recent AP was elevated significantly ($p<0\ 001$) as compared with that of healthy Finnish children of the same ages (7) while the concentration of 2 81±1 36 g/l found in patients with AP nephropathy was significantly higher than that in the patients with recent uncomplicated AP ($p<0\ 0125$) However the serum IgM levels of patients with AP nephropathy in the initial stage of recent AP (1 22±0 83 g/l) were not higher than those in the patients with recent AP as a whole In 3 patients with elevated IgM at the initial stage of nephropathy the level of IgM was normal when measured 1–3 years later

IgD was present in the serum of 11 out of the 18 patients studied in this respect The serum levels of IgE and IgD showed no significant difference as compared with those reported in healthy Swedish children (3) nor were any differences found between the AP subgroups

DISCUSSION

Previous studies have demonstrated that the serum IgA concentration is elevated in AP (8 15 16) and it seems evident from the pres

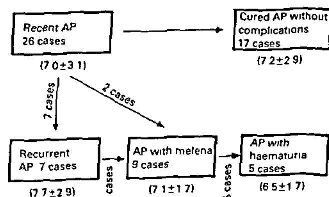


Fig 1 Outcome of anaphylactoid purpura (AP) in 26 patients Mean age in each group is given in parentheses

Acta Pædiatr Scand 66 541–548 1977

PITUITARY THYROID RESPONSIVENESS TO THYROTROPIN RELEASING HORMONE IN PRETERM AND SMALL FOR GESTATIONAL AGE NEWBORNS

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ABSTRACT Jacobsen B B Andersen H Dige Petersen H and Hummer L (University Clinic of Paediatrics Children s Hospital Fuglebakken and Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark) Pituitary thyroid responsiveness to thyrotropin releasing hormone in preterm and small for gestational age newborns Acta Paediatr Scand 66 541 1977 —A dose of 40 μg TRH was injected intravenously in 12 preterm (PT) and 15 small for gestational age (SGA) babies (with advanced gestational ages) between 5 and 167 hours after birth Serum thyrotropin (TSH) was measured prior to and 30 and 180 min after TRH serum thyroxine (T) and serum triiodothyronine (T) were measured prior to and 180 min after TRH The percentage increase in serum TSH in PT and SGA babies was comparable to that of fullterm newborns The serum TSH 30 min after TRH in SGA newborns was significantly correlated to basal TSH values such a correlation could not be shown in the preterms One SGA and four PT babies had a repeat TRH test performed later in infancy In all but one PT with a gestational age of 27 weeks the TSH rise was lower than in the neonatal period The thyroid hormone responses after TRH were similar in the two groups of babies The percentage increase above basal levels were Median serum T_3 increase about 46% and median serum T_4 increase about 14% It is concluded that in low birth weight newborn babies the pituitary TSH response to exogenous TRH was like that detected in fullterm newborns and more pronounced than later in infancy The effect of endogenous TSH as measured by thyroid hormone increases was of the same magnitude as observed in fullterms and in adults

KEY WORDS Thyrotropin thyrotropin releasing hormone thyroid hormones preterm small for gestational age newborns

The anatomical and functional maturation of the hypothalamus and pituitary gland is supposed to occur at mid gestation At this time fetal serum concentrations of thyrotropin (TSH) and other pituitary hormones increase (13 17 18 19) Thyrotropin releasing hormone (TRH) seems however to be present in fetal brain even earlier (33)

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The mechanisms underlying these changes in serum concentrations of TSH and thyroid

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Submitted Oct 1 1976
Accepted March 2 1977

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Acta Pædiatr Scand 66 541–548 1977

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The mechanisms underlying these changes in serum concentrations of TSH and thyroid

hormones are not clear (11 12) An increased endogenous TRH secretion during the first postnatal days has been suggested in fullterms (12 22)

In order to evaluate the pituitary and thyroid gland responsiveness in preterm and small for gestational age babies during the early neonatal period measurements of serum TSH and thyroid hormone concentrations were performed after exogenous TRH stimulation

MATERIALS AND METHODS

Studies were carried out in 27 euthyroid newborn low birth weight infants between 5 and 167 hours after birth (Tables 1 and 2) The parents were informed and consent obtained Twelve preterm (PT) babies of 27 to 36 weeks gestation and with weights appropriate for dates and 15 small for gestational age (SGA) babies were included in the study (Tables 1 and 2) All SGA babies—except patient no 26—were born at term (gestational age 37 to 40 weeks) Gestational age was assessed according to Bjerkedal et al (2) Infants with birth weight below the 10th percentile and with typical clinical signs were defined as SGA babies (11) Two of the SGA babies—nos 14 and 23—were perinatally asphyxiated with apgar scores 0/1 min 1/2 min 3/3 min and 4/1 min 6/5 min 9/10 min respectively but were in a good clinical state when tested Many of the infants were placed in incubators all had normal body temperature when studied The body weight in PT babies ranged from 1 155 to 2 350 g and in SGA babies from 1 370 to 2 630 g The body weights in the two groups did not differ statistically ($p>0$ 1) If the SGA infants nos 13 14 and 15 were excluded the distribution of post natal ages was approximately equal in both groups

In order to eliminate any possible influence of a circadian rhythm of TSH (31) all studies were started in the morning between 8 30 and 9 00 a m Synthetic TRH —pyroglutamyl histidyl proline amide (Hoechst AG)—40 μg was administered intravenously Blood was centrifuged immediately and serum stored at −20°C until analysed No side effects were seen The patients were included in a later prospective study So far 5 patients have been restudied (Table 3)

Serum concentrations of TSH were measured prior to and 30 min and 180 min after TRH injection In a preliminary study no significant increase in thyroid hormone concentration was observed during the first 30 min after TRH for which reason only basal values and serum concentrations 180 min after TRH were determined

Serum TSH was measured by double antibody RIA technique (8) The results are expressed in terms of mU of research Standard A The detection limit of the assay is calculated to be 0 2 mU/l serum The intra assay coefficient of variation is 6% at levels from 2 5 to 25 mU/l and 12% at levels lower than 2 5 mU/l and higher than 25 mU/l The inter assay coefficient of variation is 16% at levels from 2 5 to 10 mU/l 6% from 10 to 25 mU/l and 25% at levels lower than 2 5 mU/l and higher than 25 mU/l based on a weekly set up during one year

Serum thyroxine (T_4) was measured using a modification of the competitive binding technique (Tetralute Ames) reported by Braverman et al (3) The detection limit of the assay is 25 nmol/l The intra assay coefficient of variation is 4% in the range 77–129 nmol/l and 6–10% outside this range The inter assay coefficient of variation is 9% at levels higher than 129 nmol/l 6% at levels between 77 and 129 nmol/l and 11% for values below 77 nmol/l

Serum triiodothyronine (T_3) was measured radioimmunologically as described by Gharib et al (16) The detection limit is 0 10 nmol/l and the intra assay and inter assay coefficient of variation is 5% in the range 1 10 to 2 40 nmol/l and 8% at levels lower and higher than these values Normal range of serum TSH T_4 and T_3 for newborns of various maturity during the first 6 days of life has been reported elsewhere (23) All analyses were performed in duplicate

Calculations of median and Spearman s coefficient of rank correlation (*R*) followed standard statistical methods (7) Further the Wilcoxon test for two samples and the Wilcoxon test for pair differences were employed (7) In the calculations values below the sensitivity limit of the TSH assay are expressed as 0 1 mU/l

RESULTS

Basal values of serum TSH and thyroid hormones

The basal values of *serum TSH* seemed to be higher in SGA than in PT babies (Tables 1 and 2) and the difference could probably not be ascribed to differences in post natal age After 72 or 96 hours all basal values were within the normal range of adults The basal *thyroid hormone* concentrations in serum tended to be higher in SGA than in PT babies but did not differ statistically ($p>0$ 1) The higher basal values of serum T_4 correlated significantly with the higher serum T_3 levels in PT ($r=0$ 73 $p<0$ 05) and in SGA ($r=0$ 70 $p<0$ 05) babies Obviously there was no relationship between basal values of serum TSH and thyroid hormones

Serum TSH 30 min after TRH injection

After administration of TRH a significant rise in serum TSH concentration occurred in all

Table 1 *Clinical data and effect of 40 μg of i v TRH on serum concentrations of TSH and thyroid hormones in preterm newborn babies*

Case no	Sex	Birth weight (g)	Gestational age (weeks)	Diagnosis	Postnatal age (hours)	Body weight (g)	TRH stimulation test: Serum TSH (mU/l)	Serum T_4^a (nmol/l)	Serum T_3^b (nmol/l)
1	♀	2 400	36	PT	17	2 3[illegible]0	13 0 27 13 0	147 – –	1 49 2 63
2	♀	2 300	36	PT	18	2 350	<0 2 13 4 –	180 – 190	1 19 1 46
3	♀	2 400	36	PT	24	2 300	3 1 15 6 3 1	257 – 299	2 10 3 48
4	♀	2 000	33	PT	38	1 990	2 1 15 1 7 2	224 – 263	1 69 2 41
5	♂	2 240	35	PT hypospadia	48	2 085	1 0 18 1 5 0	157 – 185	1 28 2 29
6	♂	2 050	34	PT icterus neonat	53	1 910	1 6 36 15 8	1[illegible]1 – 121	0 89 1 07
7	♀	2 300	34	PT	64	2 300	5 0 35 8 9	157 – 178	0 8[illegible] 1 95
8	♂	1 400	29	PT icterus neonat	69	1 400	2 2 20 9 11 6	190 – –	1 17 1 69
9	♂	2 100	35	PT	106	2 190	0 3 21 9 2 1	142 – 180	0 83 2 10
10	♀	2 150	36	PT icterus neonat	113	2 095	4 9 9 8 5 0	198 – 206	– 2 74
11	♀	2 150	35	PT icterus neonat	113	2 150	<0 2 3 6 <0 2	142 – 162	1 49 1 80
12	♂	1 210	27	PT respiratory distress syndr	167	1 155	<0 2 11 6 4 5	51 – 67	0 61 –

Serum TSH time 0 30 and 180 min after TRH injection
Serum T and $-T_3$ time 0 and 180 min after TRH injection

subjects (Tables 1 and 2) The median increase in serum TSH was 7 times in PT and 4–5 times in SGA babies (Fig 1) and the 30 min TSH values in PT and SGA babies did not differ statistically ($p>0\ 05$) In SGA newborns the higher 30 min values of TSH correlated with the higher basal TSH values ($r=0\ 67$ $p<0\ 02$) whereas in PT babies—who had lower basal values—the correlation was not significant ($r=0\ 51$ $p>0\ 05$) In the asphyxiated babies a normal TSH response appeared

The dose of TRH per kg body weight ranged from 15 2 to 34 6 μg but the TSH increase was not correlated to the TRH dose The serum TSH increase *within* the maturity groups was not correlated to head circumfer

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							27	–	
							13 0	–	2 63
2	♀	2 300	36	PT	18	2 350	<0 2	180	1 19
							13 4	–	
							–	190	1 46
3	♀	2 400	36	PT	24	2 300	3 1	257	2 10
							15 6	–	
							3 1	299	3 48
4	♀	2 000	33	PT	38	1 990	2 1	224	1 69
							15 1	–	
							7 2	263	2 41
5	♂	2 240	35	PT hypospadia	48	2 085	1 0	157	1 28
							18 1	–	
							5 0	185	2 29
6	♂	2 050	34	PT icterus neonat	53	1 910	1 6	121	0 89
							36	–	
							15 8	121	1 07
7	♀	2 300	34	PT	64	2 300	5 0	157	0 82
							35	–	
							8 9	178	1 95
8	♂	1 400	29	PT icterus neonat	69	1 400	2 2	190	1 17
							20 9	–	
							11 6	–	1 69
9	♂	2 100	35	PT	106	2 190	0 3	142	0 83
							21 9	–	
							2 1	180	2 10
10	♀	150	36	PT icterus neonat	113	2 095	4 9	198	–
							9 8	–	
							5 0	206	2 74
11	♀	2 150	35	PT icterus neonat	113	2 150	<0 2	142	1 49
							3 6	–	
							<0 2	162	1 80
12	♂	1 210	7	PT respiratory distress syndr	167	1 155	<0 2	51	0 61
							11 6	–	
							4 5	67	–

Serum TSH time 0 30 and 180 min after TRH injection
Serum T and $-T_3$ time 0 and 180 min after TRH injection

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In order to eliminate any possible influence of a circadian rhythm of TSH (31), all studies were started in the morning between 8.30 and 9.00 a.m. Synthetic TRH—pyroglutamyl histidyl proline amide (Hoechst AG)—40 µg was administered intravenously. Blood was centrifuged immediately and serum stored at −20°C until analysed. No side effects were seen. The patients were included in a later prospective study. So far 5 patients have been restudied (Table 3).

Serum concentrations of TSH were measured prior to and 30 min and 180 min after TRH injection. In a preliminary study no significant increase in thyroid hormone concentration was observed during the first 30 min after TRH, for which reason only basal values and serum concentrations 180 min after TRH were determined.

Serum TSH was measured by double antibody RIA technique (8). The results are expressed in terms of mU of research Standard A. The detection limit of the assay is calculated to be 0.2 mU/l serum. The intra assay coefficient of variation is 6% at levels from 2.5 to 25 mU/l and 12% at levels lower than 2.5 mU/l and higher than 25 mU/l. The inter assay coefficient of variation is 16% at levels from 2.5 to 10 mU/l, 6% from 10 to 25 mU/l and 25% at levels lower than 2.5 mU/l and higher than 25 mU/l based on a weekly set up during one year.

Serum thyroxine (T_4) was measured using a modification of the competitive binding technique (Tetralute Ames) reported by Braverman et al. (3). The detection limit of the assay is 25 nmol/l. The intra assay coefficient of variation is 4% in the range 77–129 nmol/l and 6–10% outside this range. The inter assay coefficient of variation is 9% at levels higher than 129 nmol/l, 6% at levels between 77 and 129 nmol/l and 11% for values below 77 nmol/l.

Serum triiodothyronine (T_3) was measured radioimmunologically as described by Gharib et al. (16). The detection limit is 0.10 nmol/l and the intra assay and inter assay coefficient of variation is 5% in the range 1.10 to 2.40 nmol/l and 8% at levels lower and higher than these values. Normal range of serum TSH, T_4 and T_3 for newborns of various maturity during the first 6 days of life has been reported elsewhere (23). All analyses were performed in duplicate.

Calculations of median and Spearman's coefficient of rank correlation (R) followed standard statistical methods (7). Further, the Wilcoxon test for two samples and the Wilcoxon test for pair differences were employed (7). In the calculations, values below the sensitivity limit of the TSH assay are expressed as 0.1 mU/l.

RESULTS

Basal values of serum TSH and thyroid hormones

The basal values of *serum TSH* seemed to be higher in SGA than in PT babies (Tables 1 and 2), and the difference could probably not be ascribed to differences in post natal age. After 72 or 96 hours all basal values were within the normal range of adults. The basal *thyroid hormone* concentrations in serum tended to be higher in SGA than in PT babies but did not differ statistically ($p>0.1$). The higher basal values of serum T_4 correlated significantly with the higher serum T_3 levels in PT ($r=0.73$, $p<0.05$) and in SGA ($r=0.70$, $p<0.05$) babies. Obviously, there was no relationship between basal values of serum TSH and thyroid hormones.

Serum TSH 30 min after TRH injection

After administration of TRH a significant rise in serum TSH concentration occurred in all

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1	♀	2 400	36	PT	17	2 3 0	13 0 27 13 0	147 – –	1 49 ? 63
?	♀	2 300	36	PT	18	2 350	<0 2 13 4 –	180 – 190	1 19 1 46
3	♀	2 400	36	PT	?4	? 300	3 1 15 6 3 1	257 – 299	2 10 3 48
4	♀	2 000	33	PT	38	1 990	2 1 15 1 7 ?	224 – 263	1 69 2 41
5	♂	2 ?40	35	PT hypospadia	48	2 085	1 0 18 1 5 0	157 – 185	1 ?8 2 29
6	♂	2 0?0	34	PT icterus neonat	53	1 910	1 6 36 15 8	1?1 – 121	0 89 1 07
7	♀	2 300	34	PT	64	2 300	5 0 35 8 9	157 – 178	0 82 1 95
8	♂	1 400	?9	PT icterus neonat	69	1 400	2 2 ?0 9 11 6	190 – –	1 17 1 69
9	♂	2 100	35	PT	106	2 190	0 3 21 9 ? 1	142 – 180	0 83 2 10
10	♀	? 150	36	PT icterus neonat	113	? 095	4 9 9 8 5 0	198 – 206	– 2 74
11	♀	2 1?0	35	PT icterus neonat	113	2 150	<0 2 3 6 <0 ?	142 – 162	1 49 1 80
1?	♂	1 210	?7	PT respiratory distress syndr	167	1 155	<0 ? 11 6 4 5	51 – 67	0 61 –

Serum TSH time 0 30 and 180 min after TRH injection
Serum T_4 and $-T_3$ time 0 and 180 min after TRH injection

subjects (Tables 1 and 2) The median increase in serum TSH was 7 times in PT and 4–5 times in SGA babies (Fig 1) and the 30 *min TSH* values in PT and SGA babies did not differ statistically ($p>0\ 05$) In SGA newborns the higher 30 min values of TSH correlated with the higher basal TSH values ($r=0\ 67$ $p<0\ 02$) whereas in PT babies—who had lower basal values—the correlation was not significant ($r=0\ 51$ $p>0\ 05$) In the asphyxiated babies a nor *mal TSH* response appeared

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1	♀	2 400	36	PT	17	2 320	13 0 27 13 0	147 – –	1 49 2 63
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1	♀	2 400	36	PT	17	2 3[illegible]0	13 0 27 13 0	147 – –	1 49 2 63
[illegible]	♀	[illegible] 300	36	PT	18	2 350	<0 2 13 4 –	180 – 190	1 19 1 46
3	♀	[illegible] 400	36	PT	24	2 300	3 1 15 6 3 1	257 – 299	2 10 3 48
4	♀	2 000	33	PT	38	1 990	2 1 15 1 7 2	2[illegible]4 – [illegible]63	1 69 2 41
5	♂	2 [illegible]40	35	PT hypospadia	48	2 085	1 0 18 1 5 0	157 – 185	1 [illegible]8 2 29
6	♂	2 050	34	PT icterus neonat	53	1 910	1 6 36 15 8	1[illegible]1 – 121	0 89 1 07
7	♀	[illegible] 300	34	PT	64	2 300	5 0 35 8 9	157 – 178	0 82 1 95
8	♂	1 400	29	PT icterus neonat	69	1 400	2 2 [illegible]0 9 11 6	190 – –	1 17 1 69
9	♂	2 100	35	PT	106	2 190	0 3 21 9 2 1	142 – 180	0 83 2 10
10	♀	2 150	36	PT icterus neonat	113	2 095	4 9 9 8 5 0	198 – 206	– 2 74
11	♀	2 150	35	PT icterus neonat	113	2 150	<0 2 3 6 <0 [illegible]	14[illegible] – 162	1 49 1 80
1[illegible]	♂	1 [illegible]10	[illegible]7	PT respiratory distress syndr	167	1 155	<0 2 11 6 4 5	51 – 67	0 61 –

Serum TSH time 0 30 and 180 min after TRH injection
Serum T[illegible] and [illegible] T_3 time 0 and 180 min after TRH injection

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The dose of TRH per kg body weight ranged from 15 2 to 34 6 µg but the TSH increase was not correlated to the TRH dose The serum TSH increase *within* the maturity groups was not correlated to head circumfer

Table 2 *Clinical data and effect of 40 μg of i v TRH on serum concentrations of TSH and thyroid hormones in small for gestational age newborn babies*

Case no	Sex	Birth weight (g)	Gestational age (weeks)	Diagnosis	Postnatal age (hours)	Body weight (g)	TRH stimulation test: Serum TSH (mU/l)	Serum T_4[b] (nmol/l)	Serum T_3[b] (nmol/l)
13	♀	2 200	38	SGA	5	2 200	57 70 54	291 291	– 3 4[illegible]
14	♀	2 630	40	SGA asphyxia neonat	6	2 630	0 7 9 0 8 1	252 278	1 6[illegible] 3 15
15	♂	2 580	40	SGA	8	2 510	3 8 36 9 1	118 167	– 2 0[illegible]
16	♀	2 300	37	SGA	18	2 250	21 9 61 50	221 250	1 66 2 16
17	♀	2 200	40	SGA	22	2 200	3 4 28 17 0	293 304	2 91 4 00
18	♀	2 400	40	SGA	29	2 400	17 4 35 –	121 188	– 2 82
19	♀	1 850	40	SGA	43	1 810	11 8 41 15 6	268 288	– –
20	♂	2 150	38	SGA	65	2 060	7 6 27 14 6	293 306	– –
21	♂	2 500	40	SGA icterus neonat	66	2 590	3 8 13 5 7 2	126 131	1 0 1 28
22	♂	2 450	37	SGA caesarean sectio delivery icterus neonat	81	2 340	9 9 54 6 9	131 154	1 56 2 24
23	♂	2 600	40	SGA asphyxia neonat	81	2 590	10 0 19 8 12 8	185 211	2 01 2 32
24	♂	2 450	40	SGA gemellus A	89	2 470	0 8 42 4 2	167 219	1 40 2 62
25	♂	2 300	40	SGA gemellus B	89	2 360	<0 2 17 8 –	206 221	2 44 2 87
26	♀	1 340	34	SGA	101	1 370	5 5 20 8 5 4	157 164	– –
27	♀	1 790	39	SGA	139	1 825	1 6 9 9 0 8	178 208	1 96 3 18

Serum TSH time 0 30 and 180 min after TRH injection
[b] Serum T_4 and $-T_3$ time 0 and 180 min after TRH injection

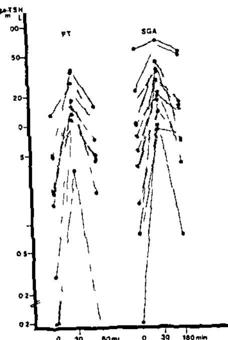


Fig 1 Serum concentration of TSH prior to and 30 and 180 min after injection of TSH 40 μg i v in preterm (PT) and small for gestational age (SGA) newborn babies (The ordinate is log serum TSH concentration)

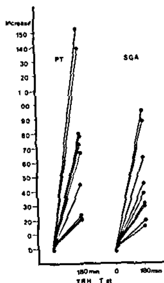


Fig 2 The increases in serum triiodothyronine (T_3) concentrations 180 min after injection of TRH 40 μg i v in preterm (PT) and small for gestational age (SGA) newborn babies (The increase is the percentage increase above basal level)

ence gestational age or basal thyroid hormone concentration

Serum TSH and thyroid hormone values 180 min after TRH injection

In most PT and SGA babies the serum concentration of TSH remained above basal level 180 min after TRH administration (PT $p<0.01$ and SGA $p<0.05$) (Fig 1)

A significant rise in serum concentrations of thyroid hormones was noted in both groups of newborns 180 min after TRH injection (Tables 1 and 2) The serum T_3 increase was invariably present and was more pronounced than the serum T_4 increase (Fig 2) A wide range of T_4 and T_3 increases was seen the median T_3 increase was about 46% in both groups and the median T_4 increase was about 14% (Fig 3) in two infants no T_4 increase could be shown No significant correlation was found between the increase in thyroid hormones and TSH response age or body weight

There was no significant difference between PT and SGA neonates ($p>0.1$)

A second TRH test later in infancy

In four PT and one SGA infants (Table 3) a second TRH test was performed 7 to 103 days after the first test It is seen that in all infants—except patient no 12 with a birth weight of 1210 g—the *TSH response* was

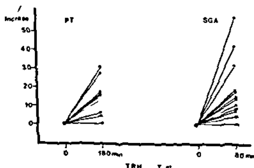


Fig 3 The increases in serum thyroxine (T_4) concentrations 180 min after injection of TRH 40 μg i v in preterm (PT) and small for gestational age (SGA) newborn babies (The increase is the percentage increase above basal level)

Table 2 *Clinical data and effect of 40 μg of i v TRH on serum concentrations of TSH and thyroid hormones in small for gestational age newborn babies*

Case no	Sex	Birth weight (g)	Gestational age (weeks)	Diagnosis	Post natal age (hours)	Body weight (g)	TRH stimulation test		
							Serum TSH (mU/l)	Serum T_4[b] (nmol/l)	Serum T_3[b] (nmol/l)
13	♀	2 200	38	SGA	5	2 200	57 70 54	291 291	– 3 42
14	♀	2 630	40	SGA asphyxia neonat	6	2 630	0 7 9 0 8 1	252 278	1 62 3 15
15	♂	2 580	40	SGA	8	2 510	3 8 36 9 1	118 167	– 2 02
16	♀	2 300	37	SGA	18	2 250	21 9 61 50	221 250	1 66 2 16
17	♀	2 200	40	SGA	22	2 200	3 4 28 17 0	283 304	2 91 4 00
18	♀	2 400	40	SGA	29	2 400	17 4 35 –	121 188	– 2 82
19	♀	1 850	40	SGA	43	1 810	11 8 41 15 6	268 289	– –
20	♂	2 150	38	SGA	65	2 060	7 6 27 14 6	293 306	– –
21	♂	2 500	40	SGA icterus neonat	66	2 590	3 8 13 5 7 2	126 131	1 0 1 28
22	♂	2 450	37	SGA caesarean sectio *delivery icterus* neonat	81	2 340	9 9 54 6 9	131 154	1 56 2 24
23	♂	2 600	40	SGA asphyxia neonat	81	2 590	10 0 19 8 17 8	185 211	2 01 2 32
24	♂	2 450	40	SGA gemellus A	89	2 470	0 8 42 4 2	167 219	1 40 2 62
25	♂	2 300	40	SGA *gemellus B*	89	2 360	<0 2 17 8 –	206 221	2 44 2 87
26	♀	1 340	34	SGA	101	1 370	5 5 20 8 5 4	157 164	– –
27	♀	1 790	39	SGA	139	1 825	1 6 9 9 0 8	178 208	1 96 3 18

Serum TSH time 0 30 and 180 min after TRH injection
[b] Serum T_4 and $-T_3$ time 0 and 180 min after TRH injection

ferences in responsiveness to TRH but also the actual TSH secretion rate at the time of study and accordingly the statistical analyses of pooled data may be subject to criticism

The TRH dose per kg body weight in the present as well as in the previous study (22) was high as compared with the doses usually given to adults (8 20 32) We found no correlation between the TRH dose per kg and the TSH response probably a maximal stimulation of the pituitary gland was obtained in both studies The serum TSH values 30 min after TRH correlated with basal TSH values in SGA babies like in fullterm newborns (22) and in euthyroid adults (32) but the responses seemed more variable in PT neonates The 30 min TSH value which is supposed to be a maximum TSH level after exogenous TRH (22) was higher during the neonatal period than later in infancy—except for the above mentioned premature infant

These findings indicate that also in low birth weight newborns—preterm babies as well as infants with advanced gestational age —the pituitary responsiveness to TRH is normal The results of this study confirm and extend previous studies (13 15 18 19 24 28) on the evolution of the pituitary-thyroid system in the fetus As low birth weight neonates are responsive to TRH this substance may be hypothesized as the initiater of the postnatal TSH hypersecretion not only in mature (23) but also in premature neonates

The increase in serum concentrations of thyroid hormones during the TRH test was probably induced by endogenous TSH The more pronounced relative increase occurred in serum T_3 concentrations a less consistent increase was observed in serum T_4 values The higher relative T_3 than T_4 responses also agree with the finding in fullterm babies that the serum T_3 increase following delivery is more pronounced than the serum T_4 rise (1 6 9 23 29) The thyroid hormone responses during serum TRH tests were although variable *not* quantitatively or qualitatively different from the responses seen in 12 fullterm newborns (unpublished results)—and not different from that seen in euthyroid adults after TRH (4 24) This is in agreement with biochemical studies of T_4/T_3 ratios in the thyroid glands in which the magnitude of the ratios were found to be the same in fetal and adult glands (14)

ACKNOWLEDGEMENTS

Skilful technical assistance was obtained from Mrs A Wilsom Mrs L Rønhof and Mrs K Jørgensen Valuable secretarial help was obtained from Miss A Jacobsen

Human TSH for iodination and anti human TSH were kindly donated by the National Pituitary Agency Bethesda USA The human TSH Research Standard A was given by the Medical Research Council Mill Hill London Synthetic thyrotropin releasing hormone was a donation from Hoechst AG The study was supported by Statens Lægevidenskabelige Forskningsfond og Dagmar Marshall Foundation

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Table 3 *The second TRH test Effect of 40 µg of i v TRH on serum concentrations of TSH and thyroid hormones*

Case no	Sex	Diagnosis	Postnatal age (days)	Body weight (g)	Serum TSH[b] (mU/l)	Serum T_4[c] (nmol/l)	Serum T_3 (nmol/l)
5	♂	PT	9	2 010	2 4 13 2 1 9	154 – 188	2 20 – 3 00
9	♂	PT	18	2 400	<0 2 3 1 <0 2	131 – 154	2 20 – 2 47
11	♀	PT	13	2 400	<0 2 2 2 <0 2	144 – 165	1 38 – 1 71
12	♂	PT	110	2 700	<0 2 19 7 2 7	170 – 178	3 30 – 4 10
19	♀	SGA	44	3 040	1 0 17 6 0 7	167 – 172	3 49 – 3 69

[a] Case no as in Tables 1 and 2
[b] Serum TSH values at time 0 30 and 180 min after TRH
[c] Serum T_4 and $-T_3$ at time 0 and 180 min after TRH

more pronounced immediately after birth The basal serum T_3 level was considerable higher at the second test in 3 of 4 patients but the relative *thyroid hormone responses* seemed to be of the same magnitude in the two tests *The TRH dose per kg body weight* ranged from 11 3 to 19 9 µg Statistical analysis was omitted because of the small number of subjects

DISCUSSION

Infants with low birth weight frequently differ from fullterm mature babies as to the pattern of postnatal growth (5 26 27 30) and for this reason *pituitary thyroid disorders* may be suspected We have elsewhere reported that a TSH hypersecretion appears in fullterm as well as in preterm and small for gestational age babies during the early neonatal period (23) The serum TSH values in the three maturity groups did not differ significantly in contrast to the thyroid hormone levels which were lower in the low birth weight babies (23) A TRH stimulation test performed in fullterm mature newborn babies demonstrated a percentage increase in serum TSH as seen in euthyroid adults in spite of high basal levels of TSH and T_4 (22) This was hypothetically interpreted as a change at the pituitary thyrotroph cell level caused by endogenous TRH secretion *in the fullterm* newborn baby

Results of TRH stimulation have however not been reported in low birth weight newborns Previous suggestions that maturation of the hypothalamic–pituitary–thyroid system is *completed at 11–24 weeks gestation* were based upon histological studies of fetal pituitary glands and on measurements of hormone concentrations in fetal blood (13 15 17 18 19 24) The present study showed that the TSH response to exogenous TRH in PT babies was of the same magnitude as observed in SGA babies and within the range of full terms (23) All children studied did respond to TRH the lowest TSH response was seen at 27 weeks gestation (no 12) The comparison of TSH responses between the groups is impeded by the non steady state of serum TSH secretion *during the first postnatal days* For this reason differences in the maximum serum TSH values after TRH not only reflect dif

Acta Pædiatr Scand 66 549–552 1977

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ABSTRACT Tovo P A and Ponzone A (Department of Paediatrics University of Turin and Regina Margherita Children s Hospital Turin Italy) Cellular and humoral factors involvement in the enhanced NBT reduction by neutrophil leucocytes of newborn infants Acta Paediatr Scand 66 549 1977.—The histochemical NBT test was performed on blood samples from ten healthy newborn infants High spontaneous NBT reduction has been confirmed for neutrophils and assessed for monocytes The stimulation of both neutrophils and monocytes with *Escherichia coli* endotoxin induces a statistically significant increase of NBT positive cells The reason for the false positi e test results in neonates was investigated by incubating neutrophils from adult donors and for different time periods in either neonatal or adult plasma before the addition of NBT NBT reduction by adult neutrophils was increased after incubation in neonatal plasma and this increase was related to the concentration of the plasma used Maximum NBT reduction was observed after 90 min of incubation at which time the NBT scores of adult cells incubated in neonatal plasma were similar to the results of tests performed on whole neonatal blood It is concluded that neonatal leucocytes demonstrate efficient spontaneous and stimulated phagocytosis and that there are in the plasma of neonates humoral factors which stimulate phagocytosis by neutrophils and are thus responsible for the false positive NBT test results observed in these subjects

KEY WORDS NBT test phagocytosis neonatal neutrophils

The nitroblue tetrazolium (NBT) test initially introduced as a rapid aid to the differential diagnosis of a pyogenic infection did not fulfill its early promise (2 7 11 14) With this test surprisingly high false positive NBT test results were demonstrated in neonates (1 3 4 7 10) The purpose of this study was to verify that NBT reduction is enhanced in neonates and to determine whether cellular or humoral factors are responsible for the enhanced dye reduction

MATERIALS AND METHODS

NBT tests

The study group consisted of 10 clinically and haematologically healthy at term infants from 8 hours to 5 days old The histochemical NBT reduction test (normal test) of Park et al (8) was modified by incubating 0 5 ml of venous blood taken into heparin (50 u/ml) with an equal amount of 0 2% NBT (Sigma) solution and phosphate buffered saline 0 15 M solution pH 7 2 in a siliconised syringe at 37°C for 15 min and then at room temperature for 10 min

For the stimulated NBT test (9) 5 µg of *Escherichia coli* endotoxin (type 055 B5 Difco) in 0 05 ml of 0 15 M phosphate buffered saline was mixed with 0 5 ml of blood in a siliconised syringe and incubated at 37 C for 10 min before the addition of the NBT whereupon subsequent steps were the same as for the normal test Thin smears were made on glass slides air-dried and stained with May-Grunwald-Giemsa s stain At least 00 cells for each subject were counted Only those cells containing a definite formazan deposit were classified as positive All counts were performed independently by two individuals and mean values for each group of experiments were calculated

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Submitted July 5 1976
Accepted March 3 1977

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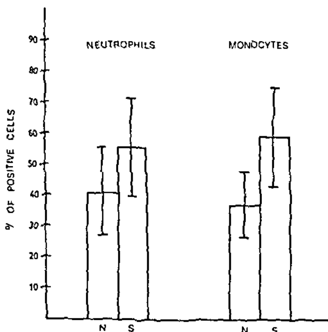


Fig 1 NBT reduction by neutrophils and monocytes of newborn infants in the normal (*N*) and stimulated (*S*) tests The mean and standard deviation of 10 tests in each group are shown

Effect of neonatal plasma on reduction of NBT by adult neutrophils

Plasma was obtained from blood from 5 neonates (umbellical cord blood) and from 5 healthy adults anticoagulated with heparin (50 u/ml) Neutrophil leucocytes were obtained from 5 healthy adults ABO blood group O negative Blood (30 ml) taken into heparin (50 u/ml) was allowed to sediment at room temperature for 2 hours The leucocyte rich supernatant was removed and centrifuged at 110 g for 10 min The leucocyte pellet was resuspended and washed twice with TC 199 medium (Difco) and adjusted to a final concentration of 20 000 neutrophils/mm³ An aliquot (1 0 ml) of this suspension was incubated with an equal volume of adult and neonatal plasma each diluted to 5% and 20% in TC 199 at 37°C Aliquots (0 2 ml) were removed after 15 30 45 60 and 90 min of incubation and NBT reduction tested with a normal NBT test

RESULTS

Results of normal and stimulated NBT test are given in Fig 1 In both the normal and the stimulated tests NBT reduction by monocytes was similar to that of neutrophil polymorphs and both were much greater than is observed in normal adults (9 11 16)

The effect of incubation of adult neutrophils in heterologous and neonatal plasma is shown in Table 1 Normal adult neutrophils incubated in heterologous adult plasma showed NBT positive scores similar to those obtained with the NBT test performed directly on adult peripheral blood When the cells were incubated in neonatal plasma NBT reduction was increased and was greater with the higher concentration of plasma After 90 min of incubation the NBT scores of adult cells were similar to the abnormally higher results obtained with neonatal blood The effect of adult plasma on the reduction of NBT by neutrophils from newborn infants was repeatedly unsuccessful because of the extreme fragility of neonatal cells which resulted in cell damage and subsequent unreliability in performing differential counts

DISCUSSION

This study confirmed that neutrophils of neonates give abnormally high results in the NBT test In addition it was shown that still more neutrophils could be induced to reduce the dye by stimulation with endotoxin This indicates that the neonate s neutrophils have a high capacity to phagocytose and reduce NBT under a normal stimulus (NBT+heparin) and a strong stimulus (endotoxin+NBT+heparin) Hence it is likely that the newborn infant possesses efficient cellular mechanisms for phagocytosis and for killing of ingested microbes

The rate of spontaneous reduction of NBT is probably due to humoral factors as shown in the second series of experiments Incubation of neutrophils of healthy adults in neonatal plasma increased the proportion of the cells that reduced the dye

Other humoral factors are capable of stimulating phagocytosis and reduction of complexed NBT in vitro and/or in vivo The serum of subjects with acute infections may enhance NBT reduction by normal neutrophil leucocytes (13) Orosomucoid one of the acute phase proteins displays a similar effect as do bacterial products including endotoxin and

Table 1 *Effect of adult and neonatal plasma upon reduction of NBT by adult neutrophils*

Mean and standard deviation of five studies

Plasma concentration	% NBT positive adult neutrophils at various time intervals of incubation (min)				
	15	30	45	60	90
Adult plasma 2 5%	6 6±4 9	7 4±3 0	6 8±4 6	15 2± 4 8	10 4± 4 5
Neonatal plasma 2 5%	13 [illegible]±6 9	10 0±5 4	13 2±7 1	23 2±10 4	35 8±11 8
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streptolysin (12 13) MIF has been shown to increase oxygen consumption and hexose monophosphate pathway activity (6) and tuftsin a tetrapeptide of splenic origin should also be regarded as a phagocytosis promoting factor (15)

Additional humoral factors may be present in the neonate Alpha feto-protein e g a polypeptide synthesized by the yolk sac and the fetal liver enhances NBT reduction when added to a suspension of normal neutrophils at a final concentration even lower than those found in the neonate (13) The NBT score of neutrophils decreases in parallel with the fall in the neonatal period of the serum concentration of this protein (5) It has been suggested that this protein may act as a *non specific* opsonin in the period preceeding the acquisition of specific immunoglobulin (13)

It may be concluded that neonate s neutrophils have efficient mechanisms of phagocytosis and metabolic changes compatible with normal intracellular bacterial killing In addition there are unidentified humoral factor(s) in neonatal plasma which promote NBT reduction by adult cells These findings are of physiological relevance and may be important as an index of normal phagocytic cell function in neonates against which phagocyte disfunction in these patients can be compared

ACKNOWLEDGEMENT

We are indebted to Dr A W Segal Clinical Research Centre Watford Road Harrow Middlesex for his advice in the preparation of this paper

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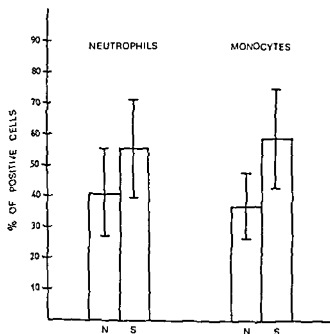


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It may be concluded that neonate s neutrophils have efficient mechanisms of phagocytosis and metabolic changes compatible with normal intracellular bacterial killing In addition there are unidentified humoral factor(s) in neonatal plasma which promote NBT reduction by adult cells These findings are of physiological relevance and may be important as an index of normal phagocytic cell function in neonates against which phagocyte disfunction in these patients can be compared

ACKNOWLEDGEMENT

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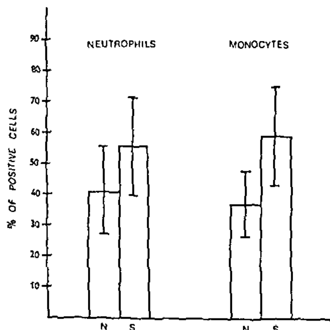


Fig 1 NBT reduction by neutrophils and monocytes of newborn infants in the normal (*N*) and stimulated (*S*) tests The mean and standard deviation of 10 tests in each group are shown

Effect of neonatal plasma on reduction of NBT by adult neutrophils

Plasma was obtained from blood from 5 neonates (umbellical cord blood) and from 5 healthy adults anticoagulated with heparin (50 u/ml) Neutrophil leucocytes were obtained from 5 healthy adults ABO blood group O negative Blood (30 ml) taken into heparin (50 u/ml) was allowed to sediment at room temperature for 2 hours The leucocyte rich supernatant was removed and centrifuged at 110 g for 10 min The leucocyte pellet was resuspended and washed twice with TC 199 medium (Difco) and adjusted to a final concentration of 20000 neutrophils/mm³ An aliquot (1 0 ml) of this suspension was incubated with an equal volume of adult and neonatal plasma each diluted to 5% and 20% in TC 199 at 37°C Aliquots (0 2 ml) were removed after 15 30 45 60 and 90 min of incubation and NBT reduction tested with a normal NBT test

RESULTS

Results of normal and stimulated NBT test are given in Fig 1 In both the normal and the stimulated tests NBT reduction by monocytes was similar to that of neutrophil polymorphs and both were much greater than is observed in normal adults (9 11 16)

The effect of incubation of adult neutrophils in heterologous and neonatal plasma is shown in Table 1 Normal adult neutrophils incubated in heterologous adult plasma showed NBT positive scores similar to those obtained with the NBT test performed directly on adult peripheral blood When the cells were incubated in neonatal plasma NBT reduction was increased and was greater with the higher concentration of plasma After 90 min of incubation the NBT scores of adult cells were similar to the abnormally higher results obtained with neonatal blood The effect of adult plasma on the reduction of NBT by neutrophils from newborn infants was repeatedly unsuccessful because of the extreme fragility of neonatal cells which resulted in cell damage and subsequent unreliability in performing differential counts

DISCUSSION

This study confirmed that neutrophils of neonates give abnormally high results in the NBT test In addition it was shown that still more neutrophils could be induced to reduce the dye by stimulation with endotoxin This indicates that the neonate s neutrophils have a high capacity to phagocytose and reduce NBT under a normal stimulus (NBT+heparin) and a strong stimulus (endotoxin+NBT+heparin) Hence it is likely that the newborn infant possesses efficient cellular mechanisms for phagocytosis and for killing of ingested microbes

The rate of spontaneous reduction of NBT is probably due to humoral factors as shown in the second series of experiments Incubation of neutrophils of healthy adults in neonatal plasma increased the proportion of the cells that reduced the dye

Other humoral factors are capable of stimulating phagocytosis and reduction of complexed NBT in vitro and/or in vivo The serum of subjects with acute infections may enhance NBT reduction by normal neutrophil leucocytes (13) Orosomucoid one of the acute phase proteins displays a similar effect as do bacterial products including endotoxin and

Table 1 *Effect of adult and neonatal plasma upon reduction of NBT by adult neutrophils*

Mean and standard deviation of five studies

Plasma concentration	% NBT positive adult neutrophils at various time intervals of incubation (min)				
	15	30	45	60	90
Adult plasma 2 5%	6 6±4 9	7 4±3 0	6 8±4 6	15 2± 4 8	10 4± 4 5
Neonatal plasma 2 5%	13 ?±6 9	10 0±5 4	13 ?±7 1	23 ?±10 4	35 8±11 8
Adult plasma 10%	9 4±4 1	13 0±7 4	8 0±4 7	14 0± 6 8	12 4± 7 6
Neonatal plasma 10%	13 2±4 8	18 4±6 3	21 6±8 7	33 6± 8 0	47 0±15 5

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Submitted Oct 18 1976
Accepted March 19 1977

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Acta Pædiatr Scand 66 553–562 1977

TRANSEPIDERMAL WATER LOSS IN NEWBORN INFANTS

I *Relation to Ambient Humidity and Site of Measurement and Estimation of Total Transepidermal Water Loss*

K HAMMARLUND G E NILSSON P Å ÖBERG and G SEDIN

From the Department of Paediatrics and the Institute of Physiology and Medical Biophysics University of Uppsala Uppsala and the Department of Medical Engineering University of Linkoping Linkoping Sweden

ABSTRACT Hammarlund K Nilsson G E Öberg P Å and Sedin G (Department of Paediatrics University Hospital Uppsala Institute of Physiology and Medical Biophysics University of Uppsala and Department of Medical Engineering University of Linkoping Sweden) Transepidermal water loss in newborn infants I Relation to ambient humidity and site of measurement and estimation of total transepidermal water loss Acta Paediatr Scand 66 553 1977.—Insensible water loss (IWL) is an important factor in the thermoregulation and water balance of the newborn infant A method for direct measurement of the rate of evaporation from the skin surface has been developed The method which is based on determination of the vapour pressure gradient close to the skin surface allows free evaporation From measurements performed on 19 newborns placed in incubators a linear relation was found between the evaporation rate (ER) and the humidity of the environment at a constant ambient temperature A 40% lower ER was recorded at a high relative humidity (60%) than at a low one (20%) in the incubator At measurements on different sites of the body a high ER was observed on the face and peripheral parts of the extremities while ER at other sites was relatively low By determining ER from different parts of the body and calculating the areas of the corresponding surfaces the total cutaneous insensible water loss for the infant in question could be obtained The transepidermal water loss (TEWL) for the whole body surface area was calculated to be 8 1 g/m²h On the basis of measurements performed it was found that the total cutaneous insensible water loss can be estimated with a reasonable degree of accuracy by recording ER from only three easily accessible measurement points

KEY WORDS Water loss water balance humidity newborn infants neonatal intensive care

The human body loses water to the environment through the skin and the respiratory passages i e by insensible water loss (IWL g/h) This water loss is an important factor for water balance and body temperature regulation Levine and co-workers (14) studied insensible water loss in infants in about 1930 and their results have constituted the basis for clinical estimation of IWL despite the fact that no infant was investigated during the first week of life

In modern neonatal intensive care disturbances of the water balance are often encountered The introduction of phototherapy treatment with radiant warmers continuous positive airway pressure and respirators has increased the demand for a thorough knowledge of the fluid requirements and thus also of fluid losses

In several investigations published in recent years the water losses from the skin and respiratory passages in newborn infants have

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Table 1 *Infant data and measurement conditions in series I*
V = vaginal delivery C S = Caesarean section S D = standard deviation

Infant	Gestational age (weeks)	Delivery	Weight at birth (kg)	Length at birth (m)	Sex	Age at start of measurement (h)	Duration of measurement (h)	T_{sk} (C) ±S D	T_{bod} (C) ±S D	T_{amb} (C) ±S D
1	39	C S	3 970	0 5 0	M	24 8	4 4	35 4±0 3	36 7±0 4	34 2±1 5
	38	C S	3 100	0 490	M	9 4	3 9	36 0±0 1	36 9±0 1	34 6±0 4
3	40	C S	2 830	0 495	F	21 1	3 3	35 7±0 2	36 7±0 1	34 8±0 2
4	40	C S	4 010	0 515	F	1 5	3 3	35 5±0 1	36 6±0 1	34 5±0 3
5	40	C S	4 430	0 555	M	1 7	2 2	34 5±0 4	36 6±0 1	34 1±0 2
6	40	C S	3 610	0 505	M	3 9	3	34 6±0 4	36 8±0 1	34 2+0 4
7	40	V	3 945	0 570	F	0 9	1 7	34 6±0 3	36 1±0 0	32 6±0 4
8	39	V	3 570	0 500	F	0 7	1 7	35 2±0 1	36 4±0 2	33 2+0 3
9	40	V	4 400	0 530	M	1 2	2 4	34 9±0 1	36 7±0 1	32 5±0 2
10	40	C S	3 770	0 500	M	1 1	1 5	35 3±0 2	36 2±0 2	33 6+0 1
11	39	C S	3 500	0 510	M	7 3	2 7	36 2±0 1	36 9±0 1	32 5±0 4
1	40	V	3 680	0 570	M	2 3	1 6	35 9±0 1	36 6±0 1	34 3±0 2
13	40	C S	850	0 4 0	F	4 5	_0	35 6±0 3	36 5±0 1	33 7±0 4
14	40	V	3 1 0	0 490	F	2 7	2 4	36 0±0 3	36 6±0 1	33 7+0 3
15	40	V	3 580	0 5 0	M	2 1	1 8	35 2±0 1	36 8±0 1	33 8±0 2
16	33	V	3 990	0 570	F	2 0	2 0	35 8±0 2	36 3±0 2	34 1±0 3
1	39	V	3 810	0 5 0	M	3 6	2 0	35 5±0 2	36 2±0 1	34 0±0 2
18	39	C S	700	0 485	F	_5	_4	35 3±0 1	36 5±0 1	32 5±0 5
19	40	C S	3 00	0 500	F	4 6	2 7	35 3±0 1	36 5±0 1	32 1±0 7
Mean	39 6		3 558	0 509		5 1	2 4	35 4	36 6	33 6
±S D	0 8		0 507	0 019		6 7	0 8	0 5	0 2	0 8

were performed When awake they were quiet and calm and showed little spontaneous motor activity The respiratory and heart rates were normal for the age

METHODS

The rate of evaporation from the skin was measured by a new method based on calculation of the vapour pressure gradient in the layer of air immediately adjacent to the skin surface (16 17 18) If this gradient is known the amount of water evaporated per unit time and area can be calculated from the equation

$$\frac{1}{A}\frac{dm}{dt} = -D\frac{\delta p}{\delta x} \qquad (1)$$

where

$\frac{1}{A}\frac{dm}{dt}$ is the amount of water evaporated per unit time and area (g/m h) in this paper expressed as evaporation rate (ER)

Table 2 *Infant data and measurement conditions in series II*
All infants were delivered by Caesarean section S D = standard deviation

Infant	Gestational age (weeks)	Weight at birth (kg)	Length at birth (m)	Sex	Age at start of measurement (h)	Duration of measurement (h)	T_{sk} (°C) range	T_{bod} (C) range	T_{amb} (C) range
1	39	735	0 475	F	0 7	6 5	34 8–36 3	36 5–37 0	33 6–36 5
	39	3 570	0 570	F	0 6	5 4	34 3–35 1	36 0–36 9	33 7–35 3
3	33	3 660	0 510	M	2 5	4 7	33 1–35 0	36 9–37 0	3 4–34 4
4	40	3 00	0 510	M	1	5 8	34 7–35 9	36 3–36 8	33 3–35 9
5	39	3 000	0 470	F	0 8	6	34 6–36 0	36 0–36 9	34 4–36 0
6	39	3 1 0	0 480	F	6 0	1 7	35 4–35 9	36 8–36 9	35 1–35 3
7	39	3 60	0 5_5	F	0 8	3 4	34 8–36 _	36 0–36 8	34 6–36 4
8	39	3 370	0 510	F	2 8	2 0	33 _–35 4	36 9–37 0	3 1–34 8
9	40	3 _30	0 500	M	1	3 1	34 4–35 1	36 3–36 8	34 2–35 0
Mean	39	3 355	0 50		1 7	3 9			
±S D	0 4	0 351	0 0__		1 7	1 8			

been determined by indirect methods, e.g. by measuring the flow and absolute humidity of the air supplied to and leaving the closed chamber in which the infant has been placed (10, 25) or by gravimetric techniques (5, 22, 23, 24). Using these methods it has been demonstrated that a relation exists between IWL and the relative humidity of the environment (10), that the water losses per surface unit are greater in infants with a low birth weight (5) and that a marked increase in the water losses takes place on exposure to radiant warmers (23).

When measuring water losses by placing the infant in a ventilated chamber it is necessary that the infant is in a good general condition and does not require nursing procedures during the period of measurement. This measuring system is slow and technical problems easily arise through the presence of urine and meconium in the measurement chamber, absorption of water in hygroscopic material, condensation in tubing etc.

Studies of water losses in very small low birth weight infants have therefore been carried out mostly by determination of the insensible weight loss (IL, g/h) with body weighing. Accurate results can only be obtained, however, when relatively long periods of measurement are used.

IWL consists partly of the total cutaneous water loss (CWL, g/h) and partly of water loss through the respiratory passages. According to Hey & Katz (10) CWL comprises about 75% of IWL. The cutaneous evaporative water loss is usually expressed per unit area and is then called transepidermal water loss (TEWL, g/m h). A selective study of TEWL in newborn infants and its relation to environmental factors, degree of activity and gestational age, for example, requires a method that allows:

- direct measurement of the rate of evaporation from the skin surface
- free evaporation from the skin surface
- quick measurements
- repeated measurements at short intervals
- measurements of relatively rapid changes
- measurement without discomfort to the infant

An instrument that satisfies the above criteria and which measures the rate of evaporation from small skin surfaces has therefore been developed for use in neonatal care and other fields.

In this paper the measurement technique employed will be briefly described. The purpose of the first study using this technique in neonatal care was to investigate the evaporation rate from the skin of newborn infants at varying degrees of ambient humidity and from different surface areas of the body. On the basis of the results obtained an easily handled way of estimating TEWL is proposed.

Abbreviations

ER	= the locally measured rate of evaporation from the surface of the skin (g/m²h)
IL	= insensible loss of weight (g/h)
IWL	= insensible loss of water (g/h)
CWL	= total cutaneous water loss (g/h)
TEWL	= transepidermal water loss (g/m²h)
Area	= area of a specified surface of the body (m²)
BSA	= total body surface area (m²)
H	= body height (m)
W	= body weight (kg)
$P_{H_2O\ amb}$	= ambient water vapour pressure (Pa)
RH_{amb}	= *ambient relative humidity (%)*
T_{amb}	= ambient temperature (°C)
T_{body}	= body temperature (°C)
T_{skin}	= skin temperature (°C)
$\frac{1}{A}\frac{dm}{dt}$	= evaporated amount of water per unit time and area (g/m²h)
D	= a constant $0.670 \cdot 10^{-3}$ (g/mhPa)
$\frac{\delta p}{\delta x}$	= vapour pressure gradient (Pa/m)
$\dot{V}O_2$	= oxygen consumption (g/h)
$\dot{V}CO_2$	= carbon dioxide production (g/h)

SUBJECTS

Measurements of ER were performed on 8 healthy newborn infants born in the 38th–40th week of pregnancy. The measurements were performed during the first 30 hours after delivery. The infant's skin was not washed or wiped before the measurements. In 19 infants (Table 1) ER was measured at varying ambient humidities (RH_{amb}) and in 9 infants (Table 2) ER was measured from different skin surfaces under constant environmental conditions. The infants were often asleep when the measurements

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V = vaginal delivery C S = Caesarean section S D = standard deviation

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15	40	V	3 580	0 5?0	M	? 1	1 8	35 2±0 1	36 8±0 1	33 8±0 2
16	39	V	3 990	0 5 0	F	? 0	2 0	35 8±0 ?	36 3±0 ?	34 1±0 3
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7	39	3 760	0 5 5	F	0 8	3 4	34 8–36 ?	36 0–36 8	34 6–36 4
8	39	3 3 0	0 510	F	8	2 0	33 –35 4	36 9–37 0	3? 1–34 8
9	40	3 30	0 500	M	1	3 1	34 4–35 1	36 3–36 8	34 2–35 0
Mean	39	3 335	0 50		1 7	3 9			
±S D	0 4	0 351	0 0		1 7	1 8			

been determined by indirect methods e g by measuring the flow and absolute humidity of the air supplied to and leaving the closed chamber in which the infant has been placed (10 25) or by gravimetric techniques (5 22 23 24) Using these methods it has been demonstrated that a relation exists between IWL and the relative humidity of the environment (10) that the water losses per surface unit are greater in infants with a low birth weight (5) and that a marked increase in the water losses takes place on exposure to radiant warmers (23)

When measuring water losses by placing the infant in a ventilated chamber it is necessary that the infant is in a good general condition and does not require nursing procedures during the period of measurement This measuring system is slow and technical problems easily arise through the presence of urine and meconium in the measurement chamber absorption of water in hygroscopic material condensation in tubing etc

Studies of water losses in very small low birth weight infants have therefore been carried out mostly by determination of the insensible weight loss (IL g/h) with body weighing Accurate results can only be obtained however when relatively long periods of measurement are used

IWL consists partly of the total cutaneous water loss (CWL g/h) and partly of water loss through the respiratory passages According to Hey & Katz (10) CWL comprises about 75% of IWL The cutaneous evaporative water loss is usually expressed per unit area and is then called transepidermal water loss (TEWL g/m²h) A selective study of TEWL in newborn infants, and its relation to environmental factors degree of activity and gestational age for example requires a method that allows

- direct measurement of the rate of evaporation from the skin surface
- free evaporation from the skin surface
- quick measurements
- repeated measurements at short intervals
- measurements of relatively rapid changes
- measurement without discomfort to the infant

An instrument that satisfies the above criteria and which measures the rate of evaporation from small skin surfaces has therefore been developed for use in neonatal care and other fields

In this paper the measurement technique employed will be briefly described The purpose of the first study using this technique in neonatal care was to investigate the evaporation rate from the skin of newborn infants at varying degrees of ambient humidity and from different surface areas of the body On the basis of the results obtained an easily handled way of estimating TEWL is proposed

Abbreviations

ER	= the locally measured rate of evaporation from the surface of the skin (g/m²h)
IL	= insensible loss of weight (g/h)
IWL	= insensible loss of water (g/h)
CWL	= total cutaneous water loss (g/h)
TEWL	= transepidermal water loss (g/m h)
Area	= area of a specified surface of the body (m²)
BSA	= total body surface area (m²)
H	= body height (m)
W	= body weight (kg)
$p_{H_2O\ amb}$	= ambient water vapour pressure (Pa)
RH_{amb}	= ambient relative humidity (%)
T_{amb}	= ambient temperature (°C)
T_{body}	= body temperature (°C)
T_{skl}	= skin temperature (°C)
$\frac{1}{A}\frac{dm}{dt}$	= evaporated amount of water per unit time and area (g/m²h)
D	= a constant 0 670 10⁻³ (g/mhPa)
$\frac{\delta p}{\delta x}$	= vapour pressure gradient (Pa/m)
$\dot{V}_{O_2}$	= oxygen consumption (g/h)
$\dot{V}_{CO_2}$	= carbon dioxide production (g/h)

SUBJECTS

Measurements of ER were performed on 78 healthy newborn infants born in the 38th–40th week of pregnancy The measurements were performed during the first 30 hours after delivery The infant's skin was not washed or wiped before the measurements In 19 infants (*Table 1*) ER was measured at varying ambient humidities (RH_{amb}) and in 9 infants (*Table 2*) ER was measured from different skin surfaces under constant environmental conditions The infants were often asleep when the measurements

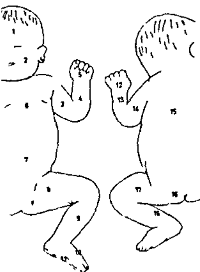


Fig 2 The measurement sites in series II

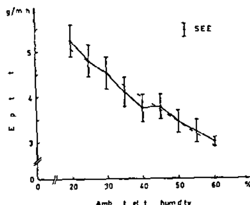


Fig 3 The relation between evaporation rate (ER) and ambient relative humidity (RH_{amb}) S E E = standard error of the estimate

65% or decreased from 60 to 0% through a change in the relation between dried and humidified air at a constant air flow Under Results values for each 5% change in RH are given At each RH level steady state was attained in the environmental factors after 5–10 min T_{amb} was kept as constant as possible (see Table 3)

II Measurement of ER from different skin surface areas
During these measurements RH_{amb} was maintained at about 50% and T_{amb} as constant as possible (see Table 4) The air supply to the incubator was kept constant at 8 l/min Measurements were performed with the infant prone supine and in the lateral position The skin surface areas examined are shown in Fig 2

TREATMENT OF DATA

The measurements gave data for ER RH_{amb} T_{sk} T_{amb} and T_{wall} In addition the vapour pressure of the ambient air ($P_{H_2O_{amb}}$) was calculated as the *product of the saturated* vapour pressure and the relative humidity which were measured in the incubator between every measurement of ER

When calculating ER for different $P_{H_2O_{amb}}$ values for ER at equidistant partial pressures (0 0 kPa) were obtained by linear interpolation between the actual measurement values for each individual infant

RESULTS

Evaporation rate at different ambient humidities

When ER was measured from an interscapular skin surface (site 15 in Fig 2) considerably higher values were obtained at a low RH_{amb} than at a high one (Fig 3) Thus ER at an RH_{amb} of 60% comprised only 60% of that measured at an RH_{amb} of 20% Measurements on six infants at an RH_{amb} of 65% gave even lower ER values (2 7 g/m²h) A linear relation between ER and RH_{amb} was obtained The coefficient of correlation between ER and RH_{amb} was −0 986 When ER was related to $P_{H_2O_{amb}}$ an essentially similar correlation was found (Fig 4) In this case the correlation coefficient was −0 971 Both modes of presentation have been chosen as RH is clinically the most commonly used parameter while $P_{H_2O_{amb}}$ is the most correct physically The

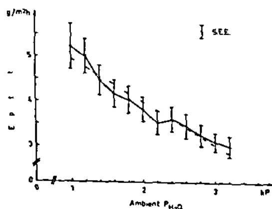


Fig 4 The relation between evaporation rate (ER) and ambient vapour pressure ($P_{H_2O_{amb}}$) S E E = standard error of the estimate

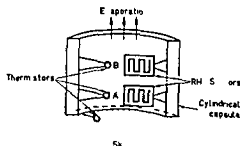


Fig 1 The arrangement of the sensors

D is a constant (0 670×10 ³ g/mhPa)
$\frac{\delta p}{\delta x}$ is the vapour pressure gradient of the water vapour (Pa/m)

This equation is valid in the immediate vicinity of the eva porative surface i e in the zone of diffusion which is about 10 mm wide At a constant rate of evaporation the vapour pressure in the diffusion zone decreases linearly with the distance from the skin surface

The vapour pressure gradient is therefore proportional to the difference in vapour pressure at two separate points (*A* and *B* in Fig 1) located on a line perpendicular to the evaporative surface The vapour pressure at the two measurement points is calculated as the product of the relative humidity and the saturated vapour pressure the latter being a function of the temperature alone According to equation 1 the water evaporation per unit time and area (ER) can then be calculated as a constant multiplied by the measured difference in vapour pressure between the two measurement points The arrangement of the different sensors over the skin section in question is illustrated in Fig 1

The relative humidity at each measurement point was measured by a small capacitive sensor of thin film type (Vaisala Humicap HMP11 Vaisala Oy Helsinki Finland) This sensor in addition to having adequate rapidity showed good linearity as well as long term stability

The saturated vapour pressure was calculated from the temperature at each measurement point As a temperature sensor a small fast thermistor was used (M81 Siemens AG Furth West Germany)

In order to maintain a stable zone of diffusion the measurement area was protected against circulating air currents with a teflon cylinder which could easily be withdrawn and sterilized

The vapour pressures in question and the difference between them were calculated in a specially constructed instrument (16 18) To obtain a stable ER value the signal was averaged in a low pass filter with a variable time constant

The instrument was supplied with a digital display for three digit read out Two ranges (00 0–99 9 and 000–999 g/m²h) could be selected Alternatively the relative humidity saturated vapour pressure and skin or air temperature could be displayed

The RH sensors were calibrated by placing them in air with known relative humidities produced in sealed boxes half filled with different saturated salt solutions (1)

The temperature of the skin area in question (T_{ski}) was measured with a separate thermistor (M81) attached to the teflon cylinder (Fig 1) but was also checked by a thermistor thermometer (YSI Model 43 Yellow Springs Ohio USA) The body temperature (T_{body}) was measured intermittently with a rectal thermometer The ambient temperature (T_{amb}) was measured in the middle of the incubator with a thermistor (M81) ER RH_{amb} T_{amb} and T_{ski} were registered in a recording system (Hokushin Electric Works Ltd Tokyo Japan)

MEASUREMENT PROCEDURE

All measurements were performed as described above with the newborn infant placed in an incubator (AGA MK41 AGA Medical Lidingo Sweden) At measurements with an air flow velocity meter (Type 8500 Thermo-anemometer Alnor Illinois USA) it was found that the air flow velocity inside the incubator was always below 15 cm/s The influence of this velocity on ER was tested by simultaneous measurements of the rate of evaporation by the described method and determination of the loss in weight of an evaporative salt solution placed on a balance inside the incubator When the fan of the incubator was turned off the weight loss of the salt solution per unit of time was reduced by 29% At the same time the measured ER decreased by 26% Consequently the air flow in the incubator caused only a small error of approximately 3% at measurements of ER from the skin surface of infants placed in an incubator when the fan was in function

The degree of influence of a low air flow velocity on the water loss from an evaporative surface found in this study is consistent with results of other investigators (20)

During the measurements T_{ski} T_{body} and T_{amb} were maintained within narrow limits T_{body} was 36 0–37 0°C in all cases This excluded sweating which according to Hey & Katz (10) does not occur at body temperatures below 37 2 C For all manipulations in the incubator during the measurement procedure the hands of the investigator were placed in airtight plastic bags carefully fixed in the openings of the incubator In this way exchange of air with air outside the incubator could be avoided during the measurement sequences

1 Measurement of ER at different ambient humidities

The infant was placed in an incubator in which the relative humidity could be varied by means of the humidifying system of the incubator and a dehumidifying system in which the air was dried by water absorbing lithium chloride (Munters Torkar AB Sollentuna Sweden) The flow of the dried air was checked with a respirometer (Wright Respirometer BOC Ltd Pinnacles UK) A total air supply (partly through the gas system of the incubator and partly through the dehumidifying system) of 15 l/min was used The dried air entered the incubator at a point immediately adjacent to the point of entry of air from the ordinary gas system of the incubator

During intermittent measurement of ER from an interscapular skin surface RH_{amb} was increased from 20 to

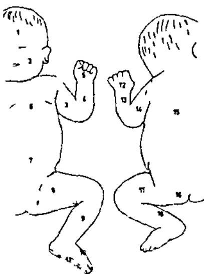


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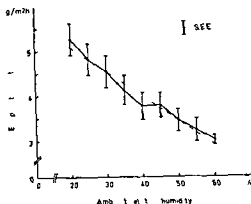


Fig 3 The relation between evaporation rate (ER) and ambient relative humidity (RH_{amb}) S E E = standard error of the estimate

65% or decreased from 60 to 0% through a change in the relation between dried and humidified air at a constant air flow Under Results values for each 5% change in RH are given At each RH level steady state was attained in the environmental factors after 5–10 min T_{am} was kept as constant as possible (see Table 3)

II Measurement of ER from different skin surface areas
During these measurements RH_{amb} was maintained at about 50% and T_{amb} as constant as possible (see Table 4) The air supply to the incubator was kept constant at 8 l/min Measurements were performed with the infant prone supine and in the lateral position The skin surface areas examined are shown in Fig

TREATMENT OF DATA

The measurements gave data for ER RH_{amb} T_{ski} T_{am} and T_{inc} In addition the vapour pressure of the ambient air ($P_{H_2O_{amb}}$) was calculated as the product of the saturated vapour pressure and the relative humidity which were measured in the incubator between every measurement of ER

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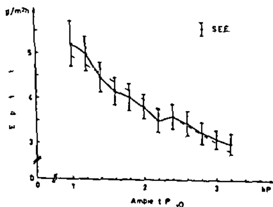


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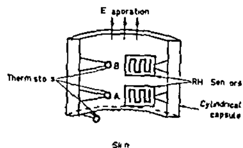


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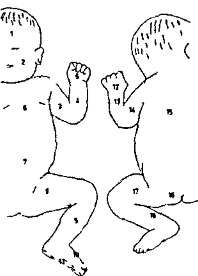


Fig 2 The measurement sites in series II

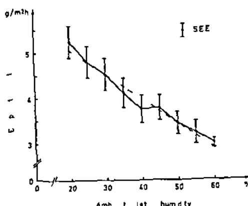


Fig 3 The relation between evaporation rate (ER) and ambient relative humidity (RH_{amb}) S E E =standard error of the estimate

65% or decreased from 60 to 0% through a change in the relation between dried and humidified air at a constant air flow Under Results values for each 5% change in RH are given At each RH level steady state was attained in the environmental factors after 5–10 min T_{amb} was kept as constant as possible (see Table 3)

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During these measurements RH_{amb} was maintained at about 50% and T_{amb} as constant as possible (see Table 4) The air supply to the incubator was kept constant at 8 l/min Measurements were performed with the infant prone supine and in the lateral position The skin surface areas examined are shown in Fig 2

TREATMENT OF DATA

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When calculating ER for different $P_{H_2O_{amb}}$ values for ER at equidistant partial pressures (0 0 kPa) were obtained by linear interpolation between the actual measurement values for each individual infant

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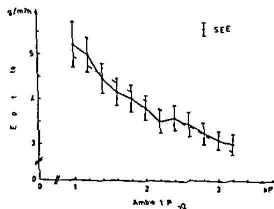


Fig 4 The relation between evaporation rate (ER) and ambient vapour pressure ($P_{H_2O_{amb}}$) S E E =standard error of the estimate

Table 3 *Skin temperature (T_{skin}) body temperature (T_{body}) and ambient temperature (T_{amb}) at different ambient relative humidities (RH_{amb})*

S D = standard deviation

RH_{amb} %	20	30	40	50	60
T_{skin} (°C) ± S D	35.4 ± 0.6	35.4 ± 0.6	35.5 ± 0.5	35.5 ± 0.4	35.4 ± 0.5
T_{body} (°C) ± S D	36.5 ± 0.3	36.5 ± 0.3	36.6 ± 0.3	36.6 ± 0.3	36.6 ± 0.3
T_{amb} (°C) ± S D	33.7 ± 1.0	33.7 ± 0.7	33.6 ± 0.8	33.5 ± 0.8	33.5 ± 0.9

regression line ( ) has been drawn in both Fig 3 and Fig 4 Regressions of a higher order deviated very little from the linear function (Fig 4)

$$ER = -0.96\, P_{H_2O} + 5.90 \qquad (2)$$

This equation is based on measurements performed during both stepwise increases and stepwise decreases of P_{H_2O}

Changes in the water content of the incubator air did not give rise to changes in the skin and rectal temperatures or in the ambient temperature (Table 3)

Evaporation rate from different body surface areas

Measurements of ER were performed at 18 sites on the skin surface (Fig 2) selected so that all parts of the body were represented ER was particularly high on the forehead (site 1) and the palm of the hand (site 5) but was also high on the cheek (site 2) the upper arm (site 3) and the sole of the foot (site 11) (see Fig 5)

The areas of skin representing the largest proportion of the total body surface area gave lower ER values On the chest (site 6) and abdomen (site 7) ER were thus 5.2 and 3.8 g/m h on an interscapular area (site 15) the value was 8.2 g/m h and on the gluteal region (site 16) 9.1 g/m²h

During the measurements of ER at different sites on the body surface T_{body} T_{amb} and RH_{amb} were carefully controlled (Table 4) As seen in the table the regional differences in skin temperature were small

Table 4 *Skin temperature (T_{skin}) body temperature (T_{body}) ambient temperature (T_{amb}) ambient relative humidity* (RH_{amb}) *and ambient vapour pressure ($P_{H_2O\,amb}$) for each measurement site in series II*

S D = standard deviation

Site of measurement	1	2	3	4	5	6	7	8	9	10	11	12
T_{skin} (°C)	35.0	35.0	34.9	34.9	34.5	34.9	35.0	34.7	34.8	34.5	34.5	34.5
± S D	0.7	0.6	0.7	0.5	0.8	0.9	0.8	1.1	1.0	0.8	0.7	0.9
T_{body} (°C)	36.7	36.6	36.8	36.8	36.8	36.9	36.9	36.8	36.8	36.9	36.7	36.8
± S D	0.4	0.4	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.3	0.2
T_{amb} (°C)	34.9	34.7	34.5	34.3	34.5	34.4	34.5	34.4	34.6	34.4	34.5	34.4
± S D	0.8	0.6	1.0	0.8	1.0	1.0	0.8	1.2	0.9	0.9	0.9	0.9
RH_{amb} (%)	51	51	52	52	52	52	51	52	51	52	52	51
± S D	2	2	3	3	2	3	2	3	3	3	[illegible]	3
$P_{H_2O\,amb}$ (kPa)	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
± S D	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2

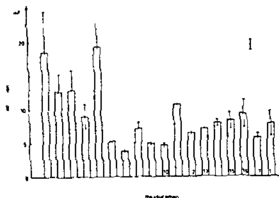


Fig 5 The evaporation rate (ER) from the 18 sites examined in series II (see Fig)

Estimation of the transepidermal water loss (TEWL)

By calculating the different areas of the body surface according to the linear formula of Du Bois (4 see also 2) and inserting the measured ER (Fig 5) into the equation

$$\mathrm{TEWL} = \frac{\sum_{i}^{i} \mathrm{ER}_i \ \mathrm{Area}_i}{\mathrm{BSA}} \qquad (3)$$

the mean evaporation (transepidermal water loss TEWL g/m h) was obtained The body surface area was calculated from Du Bois length-weight formula where the proportionality constant was changed in accordance with van Graan (7)

$$\mathrm{BSA} = 0\ 2157 W^{0\ 4\ 5}\ H^{0\ 7\ 5} \qquad (4)$$

where BSA is the body surface area (m²) H is the height (m) W is the weight of the infant (kg)

Using the above described method of calculation a mean value of 8 1 g/m²h was obtained for TEWL

For all infants the correlation between the arithmetic mean of ER from different combinations of a few measurement sites and TEWL calculated from equation (3) was tested Measurement sites 6 15 and 16 (Fig 2) gave a correlation coefficient of 0 945 (Fig 6) These sites represent a considerable proportion of the total body surface area Thus TEWL can be estimated with reasonable accuracy by measuring ER at a small number of sites using the equation

$$\mathrm{TEWL} = 0\ 92\,\overline{\mathrm{ER}}_{(6\ 15\ 16)} + 1\ 37 \qquad (5)$$

where $\overline{\mathrm{ER}}_{(6\ 15\ 16)}$ is the arithmetic mean of ER measured at three sites on the skin surface (sites 6 15 16) The total evaporative water loss from the entire skin surface (cutaneous water loss CWL g/h) is calculated as follows

$$\mathrm{CWL} = \mathrm{TEWL} \times \mathrm{BSA} \qquad (6)$$

13	14	15	16	17	18
34 9	34 6	35 3	35 1	34 9	34 8
0 5	0 9	0 8	0 5	0 7	0 7
36 8	36 8	36 5	36 7	36 8	36 8
0	0 3	0 5	0 3	0	0 3
34 6	34 5	34 7	34 9	34 6	34 8
0	0 8	1 3	0 8	0 9	0 7
50	51	51	50	53	5
	1	[illegible]	[illegible]	[illegible]	
0 7	0 8	0 8	0 8	0 9	0 8

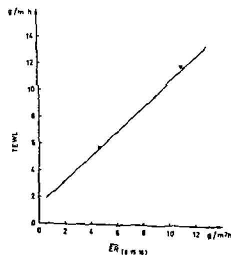


Fig 6 The relation between calculated transepidermal water loss (TEWL) and the arithmetic mean of the evaporation rate from the three skin sites 6 15 and 16

Table 3 *Skin temperature (T_{skin}) body temperature (T_{body}) and ambient temperature (T_{amb}) at different ambient relative humidities (RH_{amb})*

S D =standard deviation

RH_{amb} %	20	30	40	50	60
T_{skin} (C)±S D	35 4±0 6	35 4±0 6	35 5±0 5	35 5±0 4	35 4±0 5
T_{body} (C)±S D	36 5±0 3	36 5±0 3	36 6±0 3	36 6±0 3	36 6±0 3
T_{amb} (C)±S D	33 7±1 0	33 7±0 7	33 6±0 8	33 5±0 8	33 5±0 9

regression line (-) has been drawn in both Fig 3 and Fig 4 Regressions of a higher order deviated very little from the linear function (Fig 4)

$$ER = -0\ 96\ P_{H_2O} \quad +5\ 90 \qquad (2)$$

This equation is based on measurements performed during both stepwise increases and stepwise decreases of P_{H_2O}

Changes in the water content of the incubator air did not give rise to changes in the skin and rectal temperatures or in the ambient temperature (Table 3)

Evaporation rate from different body surface areas

Measurements of ER were performed at 18 sites on the skin surface (Fig 2) selected so that all parts of the body were represented ER was particularly high on the forehead (site 1) and the palm of the hand (site 5) but was also high on the cheek (site 2) the upper arm (site 3) and the sole of the foot (site 11) (see Fig 5)

The areas of skin representing the largest proportion of the total body surface area gave lower ER values On the chest (site 6) and abdomen (site 7) ER were thus 5 2 and 3 8 g/m h on an interscapular area (site 15) the value was 8 2 g/m h and on the gluteal region (site 16) 9 1 g/m h

During the measurements of ER at different sites on the body surface T_{body} T_{amb} and RH_{amb} were carefully controlled (Table 4) As seen in the table the regional differences in skin temperature were small

Table 4 *Skin temperature (T_{skin}) body temperature (T_{body}) ambient temperature (T_{amb}) ambient relative humidity (RH_{amb}) and ambient vapour pressure ($P_{H_2O\,amb}$) for each measurement site in series II*

S D =standard deviation

Site of measurement	1	2	3	4	5	6	7	8	9	10	11	12
T_{skin} (C)	35 0	35 0	34 9	34 9	34 5	34 9	35 0	34 7	34 8	34 5	34 5	34 5
±S D	0 7	0 6	0 7	0 5	0 8	0 9	0 8	1 1	1 0	0 8	0 7	0 9
T_{body} (C)	36 7	36 6	36 8	36 8	36 8	36 9	36 9	36 8	36 8	36 9	36 7	36 8
±S D	0 4	0 4	0 1	0 2	0 2	0 1	0 1	0 1	0 1	0 1	0 3	0 2
T_{amb} (C)	34 9	34 7	34 5	34 3	34 5	34 4	34 5	34 4	34 6	34 4	34 5	34 4
±S D	0 8	0 6	1 0	0 8	1 0	1 0	0 8	1 2	0 9	0 9	0 9	0 9
RH_{amb} (%)	51	51	52	52	52	52	51	52	51	52	52	51
±S D	2	2	3	3	2	3	2	3	3	3	2	3
$P_{H_2O\,amb}$ (kPa)	2 8	2 8	2 8	2 8	2 8	2 8	2 8	2 8	2 8	2 8	2 8	2 8
±S D	0 2	0 2	0 1	0 2	0 2	0 2	0 2	0 2	0 1	0 2	0 2	0 2

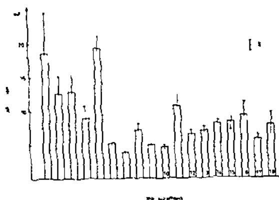


Fig 5 The evaporation rate (ER) from the 18 sites examined in series II (see Fig 2)

Estimation of the transepidermal water loss (TEWL)

By calculating the different areas of the body surface according to the linear formula of Du Bois (4 see also 2) and inserting the measured ER (Fig 5) into the equation

$$\mathrm{TEWL}=\frac{\sum_{i} \mathrm{ER}_i \ \mathrm{Area}_i}{\mathrm{BSA}} \tag{3}$$

the mean evaporation (transepidermal water loss TEWL g/m²h) was obtained The body surface area was calculated from Du Bois length–weight formula where the proportionality constant was changed in accordance with van Graan (7)

$$\mathrm{BSA}=0\ 2157W^{0\ 425}\ H^{0\ 725} \tag{4}$$

13	14	15	16	17	18
34 9 0 5	34 6 0 9	35 3 0 8	35 1 0 5	34 9 0 7	34 8 0 7
36 8 0	36 8 0 3	36 5 0 5	36 7 0 3	36 8 0	36 8 0 3
34 6 0 7	34 5 0 8	34 7 1 3	34 9 0 8	34 6 0 9	34 8 0 7
50	51 1	51	50	53	[illegible]
7 0	8 0	8 0	8 0	9 0	8 0

where BSA is the body surface area (m²) H is the height (m) W is the weight of the infant (kg)

Using the above described method of calculation a mean value of 8 1 g/m²h was obtained for TEWL

For all infants the correlation between the arithmetic mean of ER from different combinations of a few measurement sites and TEWL calculated from equation (3) was tested Measurement sites 6 15 and 16 (Fig 2) gave a correlation coefficient of 0 945 (Fig 6) These sites represent a considerable proportion of the total body surface area Thus TEWL can be estimated with reasonable accuracy by measuring ER at a small number of sites using the equation

$$\mathrm{TEWL}=0\ 92\overline{\mathrm{ER}}_{(6\ 15\ 16)}+1\ 37 \tag{5}$$

where $\overline{\mathrm{ER}}_{(6\ 15\ 16)}$ is the arithmetic mean of ER measured at three sites on the skin surface (sites 6 15 16) The total evaporative water loss from the entire skin surface (cutaneous water loss CWL g/h) is calculated as follows

$$\mathrm{CWL}=\mathrm{TEWL}\times\mathrm{BSA} \tag{6}$$

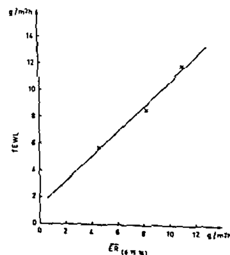


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The methods used for studies of IWL have been based either on measurement of changes in the water content of air flowing through a measurement chamber or on direct measurement of the infant s weight loss during a given period of time (IL) The former method however offers little possibility of studying infants who need nursing procedures during the relatively long measurement periods required As according to the Isenschmid equation (11) IL is not directly comparable to IWL ($IL = IWL + V_{CO} - V_O$) measurements based on weighing can give rise to an overestimation of IWL by about 10%

With the method described in this paper many of these difficulties have been overcome Direct measurement of ER has been possible without interference with normal nursing procedures Further the method has been found to satisfy demands for a high degree of accuracy a minimal influence on the examined skin surface and its ambient microclimate a short measurement time and a possibility of quickly repeated measurements The method differs in most respects from previously employed techniques for determination of the rate of evaporation from the skin surface (16)

This new method allows direct recording of changes in ER under varying external conditions (see Methods and Measurement Procedure) In the present investigation ER was studied during variations in the water content of the ambient air and a linear relation was found between ER and relative humidity at a constant ambient temperature At a high ambient humidity (60%) ER was about 60% of the value at a low humidity (20%) In previous similar investigations (10) employing indirect methods of measurement the basal transepidermal water loss was reported to be only about 30% lower at a high than at a low ambient humidity These discrepancies must be ascribed to methodological differences

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On calculation of TEWL from measured ER values a mean of 8 1 g/m h was obtained at an ambient humidity of 50% The value of 6 8 g/m²h reported by Hey & Katz (10) was obtained at an ambient humidity of about 60% Despite differences in the method of measurement and in environmental factors the results were thus fairly similar

On comparison between the ER at an interscapular skin surface (site 15) at an RH_{amb} of 50% in the measurement series I in which the ambient humidity was varied and the corresponding ER (site 15 RH_{amb} 50%) in series II a lower mean ER value was found in series I Several factors may have contributed to this difference For instance the infants examined in series I (Table 1) lay in the same position throughout the measurements while in series II (Table 2) the infants had to be turned to allow measurement on all sites depicted in Fig 2 Another factor which may have contributed to the higher ER in series II is the higher ambient temperature in that

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CONCLUSIONS

In studies of transepidermal water loss in new born infants the method described in this paper has considerable advantages

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The methods used for studies of IWL have been based either on measurement of changes in the water content of air flowing through a measurement chamber or on direct measurement of the infant's weight loss during a given period of time (IL). The former method, however, offers little possibility of studying infants who need nursing procedures during the relatively long measurement periods required. As according to the Isenschmid equation (11), IL is not directly comparable to IWL ($IL = IWL + V_{CO_2} - V_{O_2}$), measurements based on weighing can give rise to an overestimation of IWL by about 10%.

With the method described in this paper many of these difficulties have been overcome. Direct measurement of ER has been possible without interference with normal nursing procedures. Further, the method has been found to satisfy demands for a high degree of accuracy, a minimal influence on the examined skin surface and its ambient microclimate, a short measurement time and a possibility of quickly repeated measurements. The method differs in most respects from previously employed techniques for determination of the rate of evaporation from the skin surface (16).

This new method allows direct recording of changes in ER under varying external conditions (see Methods and Measurement Procedure). In the present investigation ER was studied during variations in the water content of the ambient air, and a linear relation was found between ER and relative humidity at a constant ambient temperature. At a high ambient humidity (60%) ER was about 60% of the value at a low humidity (20%). In previous similar investigations (10) employing indirect methods of measurement, the basal transepidermal water loss was reported to be only about 30% lower at a high than at a low ambient humidity. These discrepancies must be ascribed to methodological differences.

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On calculation of TEWL from measured ER values, a mean of 8.1 g/m²h was obtained at an ambient humidity of 50%. The value of 6.8 g/m²h reported by Hey & Katz (10) was obtained at an ambient humidity of about 60%. Despite differences in the method of measurement and in environmental factors, the results were thus fairly similar.

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Acta Pædiatr Scand 66 563–567 1977

PLASMA DIAZEPAM IN INFANTS AFTER RECTAL ADMINISTRATION IN SOLUTION AND BY SUPPOSITORY

FINN URSIN KNUDSEN

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ABSTRACT Knudsen F U (Department of Paediatrics Glostrup Hospital Glostrup Denmark) Plasma-diazepam in infants after rectal administration in solution and by suppository Acta Paediatr Scand 66 563 1977.—Twenty infants aged 1–2 years who had previously had one attack of febrile convulsions were randomly given a single dose of diazepam rectally either as a solution (0 7 mg/kg) or by suppository (5 mg) Plasma diazepam levels were determined repeatedly during the first hour using gas chromatography Rectal administration of diazepam in solution resulted in anticonvulsant plasma values within 4±1 min Similar plasma levels were obtained only after 20–30 min in the group treated with suppositories Diazepam in solution given rectally may therefore be useful in the acute treatment of febrile convulsions while treatment by suppository would seem to be of little value in this respect Moreover diazepam in solution given rectally seems suitable for use at home in case of recurrent febrile convulsions This treatment however cannot be recommended until the anticonvulsant effect and side-effects have been elucidated further

KEY WORDS Diazepam rectal administration plasma-diazepam anticonvulsant level febrile convulsions

The efficiency of diazepam as an anticonvulsant when administered intravenously is well documented (8 15 16 18 19 27) As this route is often unavailable especially in younger children with convulsive attacks other routes e g intramuscular (1 7 17 23) and rectal (1 2 21 23) have been attempted Thus Agurell et al (1) have recently shown that diazepam administered rectally in solution was quickly absorbed giving anticonvulsant plasma levels within minutes

The object of the present study was to determine plasma-diazepam levels following a single dose given rectally in solution or by suppository to 20 infants aged 1–2 years i e the age group with the highest incidence of febrile convulsions (14) The main purpose was to establish how fast anticonvulsant levels were achieved by the two methods

MATERIALS AND METHODS

The material (Table 1) comprised 20 infants aged 10–24 months The infants were admitted to our paediatric department with their first attack of febrile convulsions Informed consent was obtained from the parents who were later told of the results The infants were randomly allocated to one of two groups A and B Group A (10 infants) was given a suppository that contained 5 mg diazepam (Stesolid® suppository Dumex) while group B (10 infants) received a diazepam solution for rectal use (Apozepam® solution for rectal use Apothekernes Laboratorium for Specialpræparater) given with a plastic syringe connected to a 5 cm long plastic tube No attempt was made to evacuate the rectum prior to the administration Group A received a mean dose of 0 5 mg/kg (range 0 4–0 6 mg/kg) group B 0 7 mg/kg All infants were studied in a seizure free interval None had received benzodiazepine derivatives for two weeks prior to the study Ten infants (6 in group A 4 in group B) who were examined shortly after the attack of febrile convulsions had received a single dose of phenobarbital (3 mg/kg i m) 4–1 hours before The remainder who were tested in the outpatient clinic at an average of one month after the convulsions had received no anticonvulsant treat

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Submitted Nov 26 1976
Accepted Febr 23 1977

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Acta Pædiatr Scand 66 563–567 1977

PLASMA DIAZEPAM IN INFANTS AFTER RECTAL ADMINISTRATION IN SOLUTION AND BY SUPPOSITORY

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Table 3 *Plasma levels (ng/ml) after rectal administration of diazepam in solution (0 7 mg/kg)*

Case no	Min after diazepam administration					
	4±1	7±1	10±2	20±5	30±5	60±5
11	570	–	1 0 0	1 100	–	505
1	8 0	1 010	–	1 395	1 155	1 710
13	855	975	1 130	900	900	810
14	990	1 740	1 275	1 560	1 375	1 100
15	365	1 140	1 230	1 280	1 275	1 170
16	990	1 270	1 510	1 370	1 005	1 150
17	610	640	1 260	1 170	1 665	1 170
18	1 050	1 860	–	2 170	1 980	1 185
19	1 0 0	1 460	2 610	1 980	1 770	–
0	270	395	1 560	1 110	455	890
Mean	754	1 166	1 449	1 389	1 281	1 077
S E of mean	89	160	177	175	156	109

Had received phenobarbital (see text)

(Case no 17 Table 3) had febrile convulsions at a plasma level of 1 185 ng/ml which is considered far above the anticonvulsant level

DISCUSSION

The study showed that diazepam when administered rectally in solution to infants without convulsions resulted in anticonvulsant plasma levels within 4±1 min This route of administration thus seems useful in the acute treatment of febrile convulsions and a valuable alternative to the intravenous route which might be unavailable

Our results confirm Agurell and coworker s (1) observations However the age and dose range in their series was much greater than ours The present study was aimed more specifically at the age group with the highest incidence of febrile convulsions Moreover the dose of diazepam was kept more constant Our peak level (1 449 ng/ml) is higher than reported by Agurell et al (516 ng/ml) which is presumably because they used smaller doses

The study also demonstrated that in the acute situation diazepam suppositories may well be ineffective compared with the solution as anticonvulsant levels are reached very slowly (1 2 21 23) Diazepam when given rectally in solution also seems more reliable than diazepam given i m the absorption from the muscle appears unpredictable (7) and the therapeutic level is not reached until about 10 min (1) 30 min (23) or more than 60 min (17) after the i m injection

Our knowledge of the correlation between plasma level and anticonvulsant effect of diazepam is based on very few observations (1 8 26) The anticonvulsant level used (Fig 1) has therefore been chosen on a somewhat arbitrary basis Following the intravenous administration of diazepam given for seizure control there is a rapid fall of the high plasma levels (1 8 13) and convulsions may recur

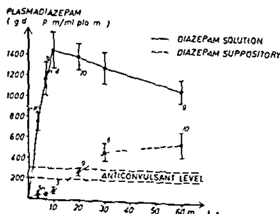


Fig 1 The mean values of plasma diazepam following administration by suppository (5 mg) (broken line) or in solution (0 7 mg/kg) (solid line) The vertical lines are S E of mean The suggested anticonvulsant plasma level of diazepam are 00–300 ng/ml n number of infants tested

Table 1 *Clinical material*

Rectal administration	No of infants	Age (month)		Weight (kg)		Diazepam doses (mg/kg)	
		Mean	Range	Mean	Range	Mean	Range
Suppository	10	15	10–22	11.0	8.1–13.5	0.5	0.4–0.6
Solution	10	17	12–24	10.9	8.7–15.5	0.7	0.7

ment for at least 3 weeks. At the time of the study 17 infants had a rectal temperature ≤37.0°C, two had 38.0–38.5 C and one had 40.0°C.

Analytical method

Blood samples (300 μl) for diazepam analysis were drawn by heel puncture in heparinized capillary blood collecting tubes. Whenever possible samples were taken at 4±1, 7±1, 10±2, 20±5, 30±5 and 60±5 min after diazepam administration. Within 1½ hours the blood was centrifuged and the plasma collected in two 50 μl micro pipets and stored at −20°C until analysed.

Diazepam and its main metabolite N desmethyl diazepam were determined in 50 μl samples of plasma by gas chromatography (4) by Apothekernes Laboratorium for Specialpræparater, Oslo, Norway. It was carried out with a 3% OV 17 column and a ^{63}Ni electron capture detector. Most samples were analysed in duplicate. The study was blind for the laboratory.

Clinical observation

The occurrence of side effects was noted.

One patient (no. 17, Table 3) developed febrile (40.0°C) universal clonic seizures and jittering lasting 4½ min. The convulsions took place 65 min after giving diazepam in solution and was observed by the author.

Statistical method

p Values were obtained by means of the Student's *t* test.

RESULTS

Plasma levels of diazepam are shown in Table 2 and 3 and also illustrated in Fig. 1.

Following the administration of 5 mg of diazepam as a suppository the anticonvulsant plasma level (probably in the region 200–300 ng diazepam/ml plasma (1, 8, 26)) was achieved after 20–30 min (Table 2). After one hour a mean plasma level of 564 ng/ml was reached (Fig. 1, Table 2).

Following rectal administration of diazepam in solution anticonvulsant plasma levels were reached in all 10 infants within 4±1 min (Fig. 1, Table 3). At that time the mean plasma value was 754 ng/ml. The mean peak level (1 449 ng/ml) was achieved within 20 min (range 1 020–2 610 ng/ml) (Table 3). Anticonvulsant levels were maintained for at least one hour (Fig. 1).

The plasma values at any point were significantly higher in the group of infants treated with diazepam in solution as compared with the group treated by suppository ($p<0.001$).

In all analyses the levels of N desmethyl diazepam in plasma were zero or less than 50 ng/ml, when corrected for the background level, which in healthy untreated adults was 50–100 ng/ml using this method.

Plasma diazepam was not influenced by previous administration of phenobarbital. This applies to the treatment by suppository ($p>0.2$) as well as solution ($p>0.2$) (Table 4).

No serious side effects were seen. The respiration appeared normal in all infants. Most became somewhat sedated and some also developed a transient ataxia. One patient

Table 2 *Plasma levels (ng/ml) after administration of 5 mg diazepam (0.4–0.6 mg/kg) by suppository*

Case no	Min after diazepam administration					
	4±1	7±1	10±2	20±5	30±5	60±5
1	30	60	–	215	245	240
2	–	45	85	370	930	1 460
3	55	50	90	220	300	900
4	–	70	–	260	445	445
5	–	–	150	110	260	325
6	90	75	–	300	310	345
7	0	50	–	185	720	355
8	–	75	–	480	–	410
9	–	0	0	–	570	455
10	70	–	125	315	–	700
Mean	49	53	90	273	473	564
S E of mean	16	8	25	36	88	117

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Table 3 *Plasma levels (ng/ml) after rectal administration of diazepam in solution (0 7 mg/kg)*

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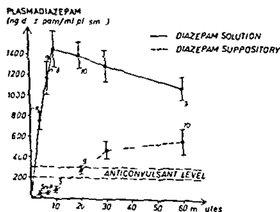


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Blood samples (300 μl) for diazepam analysis were drawn by heel puncture in heparinized capillary blood collecting tubes Whenever possible samples were taken at 4±1 7±1 10±2 20±5 30±5 and 60±5 min after diazepam administration Within 1½ hours the blood was centrifuged and the plasma collected in two 50 μl micro pipets and stored at −20°C until analysed

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p Values were obtained by means of the Student's t test

RESULTS

Plasma levels of diazepam are shown in Table 2 and 3 and also illustrated in Fig. 1

Following the administration of 5 mg of diazepam as a suppository the anticonvulsant plasma level (probably in the region 200–300 ng diazepam/ml plasma (1 8 26)) was achieved after 20–30 min (Table 2) After one hour a mean plasma level of 564 ng/ml was reached (Fig. 1 Table 2)

Following rectal administration of diazepam in solution anticonvulsant plasma levels were reached in all 10 infants within 4±1 min (Fig. 1 Table 3) At that time the mean plasma value was 754 ng/ml The mean peak level (1 449 ng/ml) was achieved within 20 min (range 1 020–2 610 ng/ml) (Table 3) Anticonvulsant levels were maintained for at least one hour (Fig. 1)

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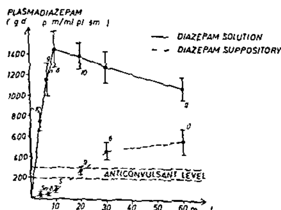


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	4±1	7±1	10±2	20±5	30±5	60±5
1	30	60	–	215	245	240
2	–	45	85	370	930	1 460
3	55	50	90	220	300	900
4	–	70	–	260	445	445
5	–	–	150	110	260	325
6	90	75	–	300	310	345
7	0	50	–	185	720	355
8	–	75	–	480	–	410
9	–	0	0	–	570	455
10	70	–	125	315	–	700
Mean	49	53	90	273	473	564
S E of mean	16	8	25	36	88	117

Had received phenobarbital (see text)

Table 3 *Plasma levels (ng/ml) after rectal administration of dia⌄epam in solution (0 7 mg/kg)*

Case no	Min after diazepam administration					
	4±1	*7±1*	*10±?*	*0±5*	*30±5*	*60±5*
11	570	–	*1 0 0*	1 100	–	505
1?	8?0	1 010	–	1 395	1 155	1 710
13	855	975	1 130	900	900	810
14	990	1 740	1 ?75	1 560	1 375	1 100
15	365	1 140	1 ?30	1 80	1 ?75	1 170
16	990	1 ?70	1 510	1 3?0	1 005	1 150
17	610	640	1 60	1 1 0	1 665	1 170
18	1 050	1 860	–	? 1?0	1 990	*1 185*
19	1 0?0	1 460	⌄ 610	1 980	1 7 0	–
?0	?70	395	1 560	1 110	455	890
Mean	754	1 166	1 449	1 389	1 ?81	1 077
S E of mean	89	160	177	1?5	156	109

Had received phenobarbital (see text)

(Case no 17 Table 3) had febrile convulsions at a plasma level of 1 185 ng/ml which is considered far above the anticonvulsant level

DISCUSSION

The study showed that diazepam when administered rectally in solution to infants without convulsions resulted in anticonvulsant plasma levels within 4±1 min This route of administration thus seems useful in the acute treatment of febrile convulsions and a valuable alternative to the intravenous route which might be unavailable

Our results confirm Agurell and coworker s (1) observations However the age and dose range in their series was much greater than ours The present study was aimed more specifically at the age group with the highest incidence of febrile convulsions Moreover the dose of diazepam was kept more constant Our peak level (1 449 ng/ml) is higher than reported by Agurell et al (516 ng/ml) which is presumably because they used smaller doses

The study also demonstrated that in the acute situation diazepam suppositories may well be ineffective compared with the solution as anticonvulsant levels are reached very slowly (1 2 21 23) Diazepam when given rectally in solution also seems more reliable than diazepam given i m the absorption from the muscle appears unpredictable (7) and the therapeutic level is not reached until about 10 min (1) 30 min (23) or more than 60 min (17) after the i m injection

Our knowledge of the correlation between plasma level and anticonvulsant effect of diazepam is based on very few observations (1 8 26) The anticonvulsant level used (Fig 1) has therefore been chosen on a somewhat arbitrary basis Following the intravenous administration of diazepam given for seizure control there is a rapid fall of the high plasma levels (1 8 13) and convulsions may recur

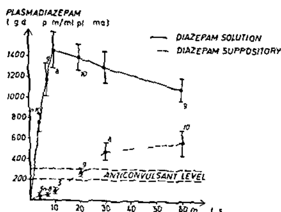


Fig 1 The mean values of plasma diazepam following administration by suppository (5 mg) (broken line) or in solution (0 7 mg/kg) (solid line) The vertical lines are S E of mean The suggested anticonvulsant plasma level of diazepam are ?00-300 ng/ml n number of infants tested

Table 4 *Influence of phenobarbital (3 mg/kg i m) on plasma levels of diazepam (mean values in ng/ml) administered by suppository and in solution*

Diazepam	Pheno barbi tal	No of pa tients	Min after diazepam administration						
			4±1	7±1	10±2	20±5	30±5	60±5	*p*
Suppository	+	6	80	55	92	293	396	447	>0 2
Suppository	−	4	28	51	88	248	549	739	
Solution	+	4	728	1 238	1 730	1 578	1 385	860	>0 2
Solution	−	6	772	1 129	1 281	1 763	1 229	1 185	

within ½–1 hour (15 18 27) We observed febrile convulsions with a plasma diazepam level of nearly 1 200 ng/ml Convulsions at a similarly high plasma level has not previously been reported

One study seems to indicate that pheno barbital may stimulate the metabolism of diazepam (26) The doses of phenobarbital used in this study did not influence the plasma levels of diazepam

The plasma values of N desmethyl diazepam was negligible because the desmethylation is a slow process in the human being (10) The anticonvulsant properties of the metabolite therefore is of no importance in acute seizure control

Diazepam administered rectally in solution seems suitable for use in recurrent febrile con vulsions and treatment may be given by the parents at home The procedure is simple and a high plasma level of diazepam is quickly reached Moreover diazepam is remarkably free from undesirable effects (6 11 12 20 24) Serious side effects (3) or death (16 19 27) have only been reported a few times and a simple cause and effect relationship could not be established in these cases (5 11 22 25)

The plasma levels reported here seem unduly high The doses should be reduced to 0 5 mg/kg if the treatment is to take place at home This would reduce the peak level but also shorten the duration of effect (9) Before the treatment can be recommended for use at home it must be investigated further with re gard to effect and side effects

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Submitted Nov ?4 1976
Accepted Febr 16 1977

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Diazepam administered rectally in solution seems suitable for use in recurrent febrile convulsions and treatment may be given by the parents at home. The procedure is simple and a high plasma level of diazepam is quickly reached. Moreover diazepam is remarkably free from undesirable effects (6, 11, 12, 20, 24). Serious side effects (3) or death (16, 19, 27) have only been reported a few times and a simple cause and effect relationship could not be established in these cases (5, 11, 22, 25).

The plasma levels reported here seem unduly high. The doses should be reduced to 0.5 mg/kg if the treatment is to take place at home. This would reduce the peak level but also shorten the duration of effect (9). Before the treatment can be recommended for use at home it must be investigated further with regard to effect and side effects.

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Acta Pædiatr Scand 66 569–571 1977

PERSISTENCE OF IN VITRO LYMPHOCYTE RESPONSE TO TUBERCULIN IN SKIN TEST NEGATIVE CHILDREN IMMUNIZED WITH BCG IN INFANCY

Z SPIRER E ASSIF V ZAKUTH N BOGAIR
J SCHWARTZ and M MENDES

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ABSTRACT Spirer Z Assif E Zakuth V and Bogair N (Paediatric Department A Rokach (Hadassah) Hospital Tel Aviv) Schwartz J (Department of Immunology Tel Aviv University Medical School Tel Aviv) and Mendes M (Y Khassis Pulmonary Diseases Prevention Centre Ministry of Health Jaffa Israel) Persistence of in vitro lymphocyte response to tuberculin in skin test negative children immunized with BCG in infancy Acta Paediatr Scand 66 569 1977.—PPD stimulated lymphocyte reactivity was tested in 119 children aged 11 to 12 years The lymphocyte responses was evaluated by measuring the extent of tritiated thymidine incorporation by cultured cells In a group of sixty four tuberculin negative children who had been BCG vaccinated in the neonatal period lymphocyte response was significantly greater than in a group of non vaccinated tuberculin negative children matched for sex The highest reactivity appeared in another group of children vaccinated in infancy and tuberculin positive Lymphocytes from BCG vaccinated children retain some sensitivity to tuberculin even years after the BCG vaccination and even at the time when the skin reactivity disappeared

KEY WORDS BCG vaccination lymphocyte response tuberculin test

Although the need and prophylactic value of BCG vaccination in control of tuberculosis is a source of controversy (1 11 12) there are countries where the policy is a routine vaccination of all infants Israeli children (excluding those in the Jerusalem area) are vaccinated routinely at the maternity hospital (4) At the age of 11 to 12 years all the children are tested with 2 PPD units and if a negative skin test is noted revaccinated (2 7)

Since a negative skin test is an accepted criterion for BCG revaccination we have therefore investigated the correlation between the skin test and the lymphocyte reactivity to PPD *in vitro* in vaccinated and non vaccinated children at this age

MATERIALS AND METHODS

Sixty four children who had been vaccinated at birth with BCG[1] were examined at the age of 11 to 12 years All of them were found to be non reacting to the tuberculin test in doses of PPD up to 50 T U In thirty nine children (Group I) the skin tests were performed before the *in vitro* studies in twenty five children (Group II) the *in vitro* study preceded the skin tests

Thirty children were non vaccinated (from Jerusalem area and of the same socio-economic status) and tuberculin negative (Group III) Twenty five children were tuberculin positive (to 5 T U) all of them having been vaccinated at birth (Group IV)

All the children were healthy with no personal or familial history of tuberculosis They were equally distributed as to sex and age

10 ml heparinized[2] blood was withdrawn from the cubital vein and allowed to sediment at room temperature

[1] Glaxo Lab BCG
[2] Heparin Fluka

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for one hour The leucocytes were separated by standard methods and cell rich plasma obtained The response was measured by the method of Valentine (14) using homologous sera in medium M E M 199 The cells (one million) were cultured with 20 μg PPD [1] with parallel blank controls and cultures with P H A [2] All cultures were done in triplicate After 72 hours of incubation 2 μc of tritiated thymidine were added to each tube Incorporation of the tritiated thymidine by the cultured cells was measured in a liquid scintillation counter and expressed in CPM/10^6 cells

RESULTS

The results are summarized in Table I In Group I the mean was 18 000±1 500 (±S E M) with a range from 6 000 to 42 000 Group II was very similar mean 18 500±1 600 (±S E M) range 6 800 to 38 000 Thus the skin testing with PPD preceding the culture had apparently no effect on the lymphocyte reactivity in this small series

In Group III non vaccinated and non reacting to tuberculin the mean value was 5 600± 1 200 (±S E M) with a range from 4 000 to 11 000

In Group IV the mean value was 32 000± 2 700 (±S E M) with a range from 12 000 to 58 000

There is a statistically significant difference ($p<0\ 05$) between Groups I and II and Group III on the one hand and Group IV on the other The difference between Groups III and IV is more significant ($p<0\ 002$)

DISCUSSION

There is evidence that lymphocyte stimulation assay may provide a sensitive and valuable indicator of exposure occult infection or a carrier state in some infectious diseases PPD is known to be a stimulator of lymphocyte transformation *in vitro* (8) It has been found active in cultures of cells from individuals with healed tuberculosis as well as those immunized with BCG

Table I *Lymphocyte response to PPD*

Groups I and II BCG vaccinated PPD-negative Group III non vaccinated PPD negative Group IV vaccinated PPD positive

Group	No of patients	Lymphocyte thymidine incorporation (CPM)	
		Mean±S E M	Range
I	39	18 000±1 500	6 000–42 000
II	25	18 500±1 600	6 800–38 000
III	30	5 600±1 200	4 000–11 000
IV	25	32 000±2 700	12 000–58 000

Generally a correlation is found between *in vitro* transformation and *in vivo* reactions to tuberculin despite wide variations in the *in vitro* results (3) Matsaniotis et al (5) found a relationship between *in vitro* response and a history of healed tuberculosis or BCG vaccination even in subjects negative to tuberculin in the skin test Another study (9) revealed that the *in vitro* response always precedes the conversion of the skin test However Thomas et al (13) found no relationship between these two phenomena in BCG vaccinated subjects

The wide range of the values as seen in our study makes the count of the PPD reactivity of lymphocytes an unreliable test in an individual examination Nevertheless when groups are examined our results show that in the vaccinated children 11 to 12 years after the BCG injection the lymphocytes still respond to PPD even when the skin reactivity had disappeared Children with a positive tuberculin reaction have the highest values of lymphocyte reactivity when stimulated with PPD

Although we cannot exclude the possible role of infection by nonpathogenic mycobacteria in maintaining a positive lymphocyte response our observations support the view that children vaccinated at birth may retain protection even though their skin reactivity has disappeared or markedly diminished It confirms the effectiveness of routine BCG vaccination of the newborn infants On the other hand it

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raises questions about the necessity of revac cination with BCG on the basis of a negative skin test only

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Submitted Sept 14 1976
Accepted March 11 1977

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In Group III non vaccinated and non reacting to tuberculin the mean value was 5600±1200 (±S E M) with a range from 4000 to 11000

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Acta Pædiatr Scand 66 573–578 1977

THE GASTROINTESTINAL ABSORPTION OF PENICILLIN V IN CHILDREN WITH SUSPECTED COELIAC DISEASE

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ABSTRACT Bolme P Eriksson M and Stintzing G (Department of Paediatrics St Goran s Hospital Stockholm Sweden) The gastrointestinal absorption of penicillin V in children with suspected coeliac disease Acta Paediatr Scand 66 573 1977.—The gastrointestinal absorption of penicillin V (pc V) was investigated in 6 children 6–12 months old with suspected coeliac disease The diagnosis was set after small bowel biopsy and absorption tests of vitamin A and *d* xylose As control groups served 7 children with diarrhoea but with normal small bowel biopsy and/or absorption tests and a group of 9 children with upper respiratory tract infection of the same ages as the children in the test group The absorption of calcium pc V in oil suspension (Penicals®) was impaired in the patients with suspected coeliac disease compared to that of the control groups There was no significantly different absorption of pc V between the control children with diarrhoea and those with upper respiratory tract infection After 6–8 months of gluten free diet in the children with suspected coeliac disease their absorptive ability of oral calcium pc V in suspension form was equal with that of a control group

KEY WORDS Coeliac disease gastrointestinal absorption penicillin V *d* xylose vitamin A small bowel biopsy

Penicillin V (pc V) remains the drug of choice when bacterial infection in the respiratory tract is suspected in children For a clinical effect it is necessary that a concentration of penicillin high enough is achieved at the site of infection

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Acta Pædiatr Scand 66 573–578 1977

THE GASTROINTESTINAL ABSORPTION OF PENICILLIN V IN CHILDREN WITH SUSPECTED COELIAC DISEASE

P BOLME M ERIKSSON and G STINTZING

From the Depa tment of Paediatrics St Goran s Hospital Stockholm Sweden

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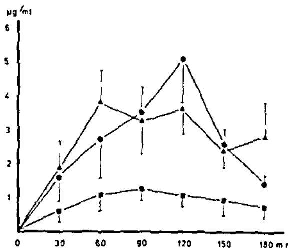


Fig 1 Plasma concentration (mean ±S E M) of penicillin after oral administration of 20 mg/kg b w of calcium penicillin V (Penicals®) in oil suspension Comparison *between control children (6–12 months)* ●—● *(n=9)* children with suspected coeliac disease ■—■ (n=6) and children with a history of diarrhoea but where the diagnosis of coeliac disease was ruled out ▲—▲ (n=7)

MATERIALS AND METHODS

Thirteen infants 6–12 months old referred to the hospital because of long lasting diarrhoea and with clinical signs of malabsorption were included in the study

On admission a careful history was taken concerning symptoms of diarrhoea and poor weight gain in relation to the introduction of gluten containing diet The time which had elapsed between introduction of gluten containing food and admission was relatively short (2–5 months) After clinical examination all children were given a test meal using simultaneous oral administration of *d* xylose glucose vitamin A and triglycerides (14) During the initial diagnostic investigations the gastrointestinal absorption of calcium pc V (Penicals®) was also determined Calcium pc V was given after at least two hours of fasting in the commercially available oil suspension in a dose of 20 mg/kg b w The absorption was judged by determining the plasma concentration of pc V at 30 min intervals for three hours When there was a strong clinical suspicion of coeliac disease or if the test meal revealed an impaired absorption of *d* xylose and/or vitamin A we performed a small bowel biopsy using the paediatric Crosby Kugler capsule *The biopsy specimen* was then examined in dissecting as well as in light microscope

The criteria for the diagnosis suspected coeliac disease were 1) clinical signs and symptoms 2) atrophy of the small intestinal mucosa i e a relative absence of villi

Following the criteria mentioned above the initial 13 children could be divided into two groups

1) Six patients with signs and symptoms suggesting the diagnosis of coeliac disease

2) Seven children with a history of diarrhoea but with a normal test meal and a normal intestinal mucosa if examined

As control group for the absorption of pc V served children who were prescribed pc V because of upper respiratory tract infection The control children were selected so that they were of the same age as the tested children None of the children in the control group had diarrhoea on the day when their pc V absorption was tested

In two infants with suspected coeliac disease the absorption of 20 mg/kg b w of calcium pc V was compared with that of 40 mg/kg b w of the same preparation

The plasma concentration of penicillin was determined using a microbiological method Ten µl of the patients plasma applied on a paper disc was put on Bacto Antibiotic Medium 5 (Difco) and the growth inhibiting capacity of *Sarcina Lutea* was determined The method has earlier been described in detail (1 7)

After the initial diagnostic measures the six infants where coeliac disease was suspected were prescribed a gluten free diet Repeated determination of the absorption of orally administered pc V was done in these six children *after 2–3 months on diet After 6–8 months they were* once again admitted for clinical examination and repeated small bowel biopsy was performed to check the histological improvement of the mucosa A test meal was given also at this occasion and the absorption of pc V was determined

RESULTS

In Fig 1 are shown the plasma concentrations of pc-V after the administration of 20 mg/kg b w of calcium pc V suspension to 1) Control patients which were children prescribed pc V because of upper respiratory tract infections 2) Children with suspected coeliac disease before diet and 3) infants admitted be

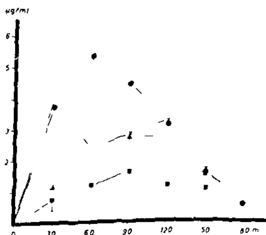


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cause of a history of diarrhoea but where the diagnosis of coeliac disease could be ruled out after diagnostic investigations

The six patients with suspected coeliac disease showed lower plasma concentrations of penicillin than the control group The differences in plasma concentrations between these two groups were statistically significant at 90 min ($p<0.01$) 120 and 150 min ($p<0.05$) The children admitted because of diarrhoea but where the diagnosis of coeliac disease could be ruled out showed no significant impairment of the absorption capacity of calcium pc V compared to the control group

The gastrointestinal absorption of calcium pc V in the six patients with suspected coeliac disease gradually improved on a gluten free diet as illustrated in Fig 2 After 6–8 months on diet the plasma concentrations of pc V were almost reaching the levels of those of the controls which were patients of the same age as the test group children after 6–8 months on diet

In Table 1 the individual children with suspected coeliac disease are listed The histological appearance of the small intestinal mucosa the absorption of vitamin A and *d* xylose in the test meal as well as the absorption of calcium pc V are presented The villous atrophy of the small intestinal mucosa is described as slight moderate or subtotal The absorption of vitamin A is classified as normal if a plasma level of 500 IU/100 ml was reached

Table 1 *Patients with suspected coeliac disease*

Small bowel biopsy vitamin A *d* xylose and penicillin V absorption

Patient	Small bowel biopsy (degree of atrophy)	Absorption tests: Vita min A	*d* xylose	Penicil lin V
HR	Moderate	Normal	Normal	Patol
CMW	Subtotal	Normal	Normal	Normal
CA	Slight	Patol	Patol	Normal
RLN	Subtotal	Patol	Patol	Patol
FS	Moderate	Patol	Patol	Patol
DH	Moderate	Patol	Patol	Normal

within 6 hours (8) The absorption of *d* xylose given in the test meal was regarded as normal if a plasma level of 20 mg/100 ml was reached within 3 hours The absorption of calcium pc V is listed as pathologic if the plasma concentration at 30–90 min was below −1 S D of the mean value for the control group As can be seen from Table 1 there was no definite correlation between degree of atrophy of the mucosa and the results of the absorption tests

Table 2 presents the same patients as in Table 1 after 6–8 months on a strict gluten free diet As can be seen three out of the six patients had a normal intestinal mucosa in two of the children the degree of atrophy of the mucosa had diminished Only one child had an unchanged mucosa This child (HR) was shown to have in addition a cow s milk protein intolerance and not until cow milk was totally excluded from the diet the intestinal mucosa showed healing

In Table 3 is shown the individual children with long lasting diarrhoea where the diagnosis of coeliac disease was ruled out As can be seen two of the seven children had a decreased absorption of pc V

By doubling the dose from 20 to 40 mg/kg b w of calcium pc V a higher plasma concentration of penicillin could be found in two children with suspected coeliac disease as shown in Fig 3

Table 2 *Patients with suspected coeliac disease*

Small bowel biopsy vitamin A *d* xylose and penicillin V absorption 6–8 months after introduction of a gluten free diet

Patient	Small bowel biopsy (degree of atrophy)	Absorption tests: Vita min A	*d* xylose	Penicil lin V
HR	Moderate	Patol	Patol	Patol
CMW	Moderate	Normal	Normal	Normal
GA	Normal	Normal	Normal	Normal
RLN	Moderate	Normal	Normal	Patol
FS	Normal	Patol	Normal	Normal
DH	Normal	–	Normal	Normal

This patient was shown to have also a cow s milk protein intolerance

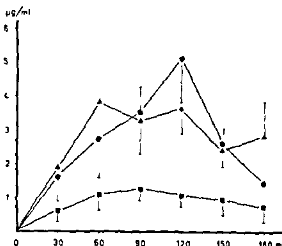


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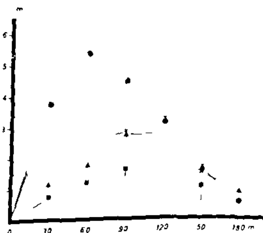


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one patient who had been on a gluten containing diet for the shortest time This patient (GA) showed only slight villous atrophy

In the test meal the absorption of *d* xylose is known to be relatively delayed and not to reach the same level as when an isolated *d* xylose absorption test is performed (10 12 14) Therefore if the plasma concentration reached 20 mg/100 ml or higher within three hours it was regarded as normal With this definition the *d* xylose absorption was pathologic in four of the six cases The vitamin A absorption was found to be normal in two cases in spite of villous atrophy Thus there was no definite correlation between the morphological findings of the small bowel biopsy and the absorption of either vitamin A *d* xylose or pc V at the initial investigation period

We could see an improvement of the histological appearance of the small intestinal mucosa after 6–8 months on a strict gluten free diet although three out of the six patients did not have a definite normalization which is known to take a longer time in some patients (17) At the same time we could see an improvement of the absorption of *d* xylose in most children There was also a definite improvement of the absorption of pc V even if it was still pathologic according to our criteria in two cases (HR and RLN) Thus the improvement of the absorptive ability seems to parallel the healing of the mucosa

The variability in the results of the absorption test especially initially at the time when the diagnosis was to be set further stresses the importance to rely more upon clinical signs and history than to the result of an absorption test in deciding whether or not to perform a small bowel biopsy in cases of suspected coeliac disease (9)

The patients in our study with suspected coeliac disease have later been challenged with a gluten containing diet and they will be followed to see if they will fulfil the criteria for coeliac disease (11) The results of this study will be reported separately

ACKNOWLEDGEMENTS

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Table 3 *Patients with long lasting diarrhoea*

Patient	Small bowel biopsy (degree of atrophy)	Absorption tests: Vitamin A	*d* xylose	Penicillin V
JL	–	–	Normal	Normal
JK	Normal	Normal	Normal	Normal
FY	Normal	Normal	Normal	Patol
MN	Normal	Patol	Patol	Normal
JN	–	Normal	Normal	Patol
JZ	–	Normal	Normal	Normal
MR	–	–	Normal	Normal

DISCUSSION

The uptake of pc V in healthy adults has been shown to take place predominantly in the proximal jejunum (5) For this reason it was plausible to believe that disorders affecting the proximal jejunum as e g coeliac disease should be associated with impaired absorption of pc-V

In this study we have shown that infants with suspected coeliac disease have a significantly impaired gastrointestinal absorption of pc V compared to a control group of infants treated for respiratory tract infection At the same time we have shown that infants with *long lasting diarrhoea but where the suspicion* of malabsorption could be ruled out did not significantly differ in the pc V absorption ability from a control group The results are in agreement with those obtained in adults where Davis & Pirola (3) found that three patients with gluten induced enteropathy verified by intestinal biopsy absorbed pc V definitely less than did normal subjects and patients with steatorrhea due to other reasons than gluten sensitivity

Parsons et al (15) studied the absorption of several drugs including pivampicillin ampicillin amoxycillin and cephalexin in adult patients with coeliac disease They concluded that the absorption of drugs in patients with coeliac disease could either be increased impaired delayed or unchanged No pattern according to physico chemical properties of the drugs could be seen in the change in the absorption of the different drugs However the study by Parsons et al (15) cannot be compared with ours since they investigated adult patients on a gluten free diet and intestinal biopsy was never performed during the study

The patients in this study have not yet fulfilled the diagnostic criteria for coeliac disease (see e g 11) which are as follows 1) clinical symptoms and histological changes of the small intestinal mucosa 2) clinical and histological improvement on a gluten free diet 3) histological and clinical relapse when gluten is reintroduced in the diet Therefore we have chosen to give our patients the diagnosis of suspected coeliac disease After introduction of a gluten free diet all patients showed normal weight gain and the gastrointestinal symptoms totally disappeared except in one case (HR) This patient showed evidence of having in addition a cow s milk protein intolerance and became clinically healthy when also cow milk was excluded from the diet

On the first admission our patients showed subtotal to moderate villous atrophy except in

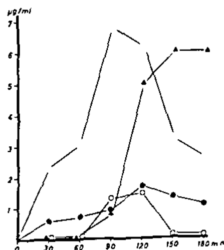


Fig 3 Plasma concentration of pencillin after oral administration of 20 mg/kg and 40 mg/kg b w of calcium pc V (Penicals®) in two patients with suspected coeliac disease before diet treatment HR 20 mg/kg b w O—O 40 mg/kg b w ×—× JS 20 mg/kg b w ●—● 40 mg/kg b w ▲—▲

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Parsons et al (15) studied the absorption of several drugs including pivampicillin ampicillin amoxycillin and cephalexin in adult patients with coeliac disease They concluded that the absorption of drugs in patients with coeliac disease could either be increased impaired delayed or unchanged No pattern according to physico chemical properties of the drugs could be seen in the change in the absorption of the different drugs However the study by Parsons et al (15) cannot be compared with ours since they investigated adult patients on a gluten free diet and intestinal biopsy was never performed during the study

The patients in this study have not yet fulfilled the diagnostic criteria for coeliac disease (see e g 11) which are as follows 1) clinical symptoms and histological changes of the small intestinal mucosa 2) clinical and histological improvement on a gluten free diet 3) histological and clinical relapse when gluten is reintroduced in the diet Therefore we have chosen to give our patients the diagnosis of suspected coeliac disease After introduction of a gluten free diet all patients showed normal weight gain and the gastrointestinal symptoms totally disappeared except in one case (HR) This patient showed evidence of having in addition a cow s milk protein intolerance and became clinically healthy when also cow milk was excluded from the diet

On the first admission our patients showed subtotal to moderate villous atrophy except in

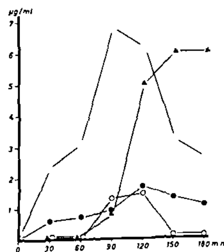


Fig 3 Plasma concentration of penicillin after oral administration of 20 mg/kg and 40 mg/kg b w of calcium pc V (Penicals®) in two patients with suspected coeliac disease before diet treatment HR 20 mg/kg b w O—O 40 mg/kg b w ×—× JS 20 mg/kg b w ●—● 40 mg/kg b w ▲—▲

Acta Pædiatr Scand 66 579–584 1977

GENETIC ENVIRONMENTAL INTERACTIONS IN PHYSICAL GROWTH

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ABSTRACT Martorell R Yarbrough C Lechtig A Delgado H and Klein R E (Division of Human Development Institute of Nutrition of Central America and Panama (INCAP) Guatemala C A) Genetic environmental interactions in physical growth Acta Paediatr Scand 66 579 1977 —Variability in stature among young children is often ascribed to health and nutrition differences in malnourished populations and to genetic differences in well nourished populations Hence it was hypothesized that parent–child correlations in malnourished Guatemalan populations would be markedly lower than those reported for European samples Instead it was found that parent–child and sibling correlations were similar in both kinds of populations The simplest interpretation of these results is that variability in stature among malnourished children is as much a reflection of genetic differ ences as in developed nations However explanations can also be advanced which would attribute the higher than expected correlations to the environment For instance it could be that socioeconomic and nutritional status is correlated across generations In other words parents who had relatively better conditions as children are more likely to provide a better environment for their children Consequently the relative contribution of genetics and envi ronment to variability in height is still unsettled Nonetheless it appears that variability in body size in malnourished populations regardless of the relative importance of its causes is a useful indicator of health and nutrition

KEY WORDS Growth genetics height preschool children

Public health officials working with malnour ished children of developing nations generally assume that variability in height principally reflects differential nutrition and health his tories (7) This belief is derived from numer ous studies indicating that chronic malnutri tion (4) and high morbidity rates (13) cause physical growth retardation

On the other hand the corresponding as sumption in industrialized nations is that vari ability in height is to a large extent a reflec tion of differences in growth potentials (19) This assumption results from large bodies of data yielding genetically predictable relation ships between parental and child stature (10)

The variables one would like to correlate in growth genetic studies are the growth poten tials of parents and children In the developed nations environmental conditions may permit full expression of the genetic potential for sta ture On the other hand in chronically mal nourished populations height of both parents and children are not full expressions of the growth potential (5) Hence stature should be a poor proxy of genetic potential in mal nourished populations and parent–child stat ure correlations would be expected to be much lower in chronically malnourished populations than in well to-do nations

This paper describes parent–child and sib ling correlations in height in data collected in four Guatemalan villages Correlations at ages from 0 5 to 7 years are presented Since chro nic protein calorie malnutrition is endemic in

17 Visakorpi J K & Savilahti E *A clinical and morphological study of the permanence of gluten intolerance* I Celiac disease E S Livingstone Ltd Edinburgh and London 1971

Submitted Nov 30 1976
Accepted March 29 1977

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The sample included 1 115 children for whom anthropometric data were available for one or more points in time and for one or both of the parents This represents 78% of the total population at risk Means and standard deviations of height from birth to 7 years of age for this sector which have been reported elsewhere (23) show rural Guatemalan children of both sexes to be substantially shorter than a well nourished reference sample from Denver Colorado (6) At birth differences are minor but later on increase until two years of age During the period comprised from ? to 5 years these differences are rather stable but then increase up to the age of 7 years when Guatemalan children are 13 cm shorter than those from Denver

The number of fathers mothers and parent pairs (i e both of them measured) as well as their mean height are given in Table 1 The percentage of fathers and mothers measured were 58% and 66% respectively In comparison to ?1 year old males from Denver (6) the fathers in our study are 19 cm shorter while the corresponding statistic for mothers is 17 cm Assortative mating for height is negligible for this population as the father–mother height correlation is 0 09 (n=260 N S) This value is lower than that of 0 3 usually reported for European populations (18 19)

For comparative purposes this paper utilizes data from samples of European descent The basic assumption is that these are healthy well nourished populations Two samples from the United States of America are included one from the longitudinal study of the Fels Research Institute in Ohio (? 3) and one from the Berkeley longitudinal study (1) The European samples include a combined sample of the London Brussels Stockholm and Zurich longitudinal studies (19) and a sample from the Finnish longitudinal study (?0) While these studies on populations of European descent do not constitute the total carried out up to date all of them are of a longitudinal character where children were carefully measured at specific ages and which included measured rather than reported parental heights

USA (Fels)		USA[b] (Berkeley)		Pool of European cities		Finland[d]	
n	r	n	r	n	r	n	r
160	0 8	?9	0 61	110	0 43	–	–
164	0 34	?6	0 67	10?	0 41	72	0 43
158	0 40	23	0 66	96	0 46	7?	0 39
152	0 21	23	0 69	88	0 47	7?	0 4?
150	0 44	23	0 60	95	0 51	69	0 50
141	0 44	24	0 60	96	0 55	69	0 51
136	0 44	?4	0 61	97	0 49	65	0 38
127	0 43	2?	0 61	96	0 49	60	0 31

RESULTS

Table 2 shows parent–child correlations for height for ages 0 5 through 7 years Father–daughter correlations appear to be the lowest with no apparent differences between father–son mother–son and mother–daughter correlations Livson et al (10) have emphasized the fact that mother–child correlations are generally higher than father–child correlations The present findings are therefore only partially in agreement with the above observation As Tiisala & Kantero (20) point out highly conflicting observations have been reported in the literature on the relative effect of paternal and maternal height on the height of sons and daughters

Correlations between mid parent heights and heights of their children are presented in Table 3 for the study sample for USA samples (1 2) and for European samples (19 20) In all of these studies mid parent height was the average of the mother s and the father s height

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Mothers			Fathers			Mid parent[a]		
n	X	S D	*n*	X	S D	*n*	X	S D
374	148.8	5.4	331	160.4	5.9	260	154.9	4.1

[a] Average of height of father and mother

Table 2 *Correlations between heights of parents and their children in rural Guatemalan data*

Age (y)	Sons				Daughters			
	Fathers		Mothers		Fathers		Mothers	
	n	*r*	*n*	*r*	*n*	*r*	*n*	*r*
0.5	186	0.30	251	0.35	155	0.19	202	0.27
1	212	0.37	262	0.33	178	0.22	227	0.22
2	251	0.35	301	0.34	207	0.18	253	0.33
3	253	0.35	299	0.32	235	0.16	275	0.31
4	244	0.33	269	0.30	224	0.24	243	0.40
5	219	0.30	231	0.30	224	0.23	240	0.40
6	199	0.31	201	0.41	206	0.19	211	0.37
7	178	0.24	178	0.42	192	0.17	186	0.38

these populations the hypothesis to be tested is that the correlations obtained in this study will be substantially smaller than those obtained in well nourished populations of European descent

METHODS

The data presented are drawn from an ongoing longitudinal study investigating the effects of chronic malnutrition on mental development and physical growth (9) Four rural ladino communities of Guatemala are being studied These villages are very poor their annual income per family being around $200 (USA) protein calorie malnutrition is endemic and morbidity rates are high

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Table 3 *Correlations between mid parent heights and heights of their children in the study sample and in USA and Europe*

Age (y)	Sons										Daughters	
	Guatemala		USA (Fels)		USA[b] (Berkeley)		Pool of European cities		Finland[d]		Guatemala	
	n	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*
0.5	182	0.38	170	0.38	32	0.35	141	0.44	–	–	152	0.31
1	202	0.44	175	0.42	29	0.39	139	0.52	55	0.45	172	0.28
2	234	0.42	166	0.45	27	0.36	131	0.49	54	0.46	197	0.34
3	236	0.44	166	0.45	26	0.44	128	0.61	52	0.51	222	0.30
4	219	0.45	162	0.48	25	0.52	134	0.57	54	0.47	200	0.40
5	187	0.45	162	0.48	23	0.50	133	0.58	53	0.43	198	0.38
6	165	0.55	160	0.49	26	0.56	129	0.49	46	0.38	171	0.32
7	145	0.53	153	0.47	24	0.54	129	0.55	42	0.36	150	0.34

Garn (2)   Tanner Goldstein & Whitehouse (19)
[b] Bayley (1)   [d] Tiisala & Kantero (20)

as to parenthood was obtained from the civil records which are believed to be highly reliable All information was collected between January 1969 and July 1975

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USA (Fels)		USA[b] (Berkeley)		Pool of European cities		Finland[d]	
n	*r*	*n*	*r*	*n*	*r*	*n*	*r*
160	0 78	49	0 61	110	0 43	–	–
164	0 34	6	0 67	107	0 41	72	0 43
158	0 40	73	0 66	96	0 46	72	0 39
157	0 71	73	0 69	88	0 47	72	0 42
150	0 44	23	0 60	95	0 51	69	0 50
141	0 44	74	0 60	96	0 55	69	0 51
136	0 44	74	0 61	97	0 49	65	0 38
177	0 43	72	0 61	96	0 49	60	0 31

RESULTS

Table 2 shows parent–child correlations for height for ages 0 5 through 7 years Father–daughter correlations appear to be the lowest with no apparent differences between father–son mother–son and mother–daughter correlations Livson et al (10) have emphasized the fact that mother–child correlations are generally higher than father–child correlations The present findings are therefore only partially in agreement with the above observation As Tisala & Kantero (20) point out highly conflicting observations have been reported in the literature on the relative effect of paternal and maternal height on the height of sons and daughters

Correlations between mid parent heights and heights of their children are presented in Table 3 for the study sample for USA samples (1 2) and for European samples (19 20) In all of these studies mid parent height was the average of the mother s and the father s height

The differences seen between the correlations in the four studies on populations of European descent (see Table 3) exemplify the range of values reported in the literature for such populations a point emphasized by Livson et al (10) who demonstrated that even pooled estimates of such parent–child correlations have wide confidence intervals at all ages

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Mothers			Fathers			Mid parent[a]		
n	X	S D	*n*	X	S D	*n*	X	S D
374	148 8	5 4	331	160 4	5 9	260	154 9	4 1

Average of height of father and mother

Table 2 *Correlations between heights of parents and their children in rural Guatemalan data*

Age (y)	Sons				Daughters			
	Fathers		Mothers		Fathers		Mothers	
	n	*r*	*n*	*r*	*n*	*r*	*n*	*r*
0 5	186	0 30	251	0 35	155	0 19	202	0 27
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3	253	0 35	299	0 32	235	0 16	275	0 31
4	244	0 33	269	0 30	224	0 24	243	0 40
5	219	0 30	231	0 30	224	0 23	240	0 40
6	199	0 31	201	0 41	206	0 19	211	0 37
7	178	0 24	178	0 42	192	0 17	186	0 38

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METHODS

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0 5	182	0 38	170	0 38	32	0 35	141	0 44	–	–	152	0 31
1	202	0 44	175	0 47	29	0 39	139	0 52	55	0 45	172	0 [illegible]8
2	234	0 42	166	0 45	27	0 36	131	0 49	54	0 46	197	0 34
3	236	0 44	166	0 45	26	0 44	128	0 61	52	0 51	222	0 30
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160	0 28	29	0 61	110	0 43	–	–
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RESULTS

Table 2 shows parent–child correlations for height for ages 0 5 through 7 years Father–daughter correlations appear to be the lowest with no apparent differences between father–son mother–son and mother–daughter correlations Livson et al (10) have emphasized the fact that mother–child correlations are generally higher than father–child correlations The present findings are therefore only partially in agreement with the above observation As Tisala & Kantero (20) point out highly conflicting observations have been reported in the literature on the relative effect of paternal and maternal height on the height of sons and daughters

Correlations between mid parent heights and heights of their children are presented in Table 3 for the study sample for USA samples (1 2) and for European samples (19 20) In all of these studies mid parent height was the average of the mother s and the father s height

The differences seen between the correlations in the four studies on populations of European descent (see Table 3) exemplify the range of values reported in the literature for such populations a point emphasized by Livson et al (10) who demonstrated that even pooled estimates of such parent–child correlations have wide confidence intervals at all ages

The correlations for the Guatemalan sample clearly do not differ from those of the European samples in the case of the mid parent–son correlations

There is some tendency for the Guatemalan mid parent–daughter correlations to be lower than those of the European samples In all cases correlations from the Fels (2) and the combined European sample (19) are higher than the mid parent–daughter correlations from the Guatemalan study Correlation from

Table 1 *Means and standard deviations for parental height (cm) in a rural Guatemalan sample*

Mothers			Fathers			Mid parent[a]		
n	X	S D	*n*	X	S D	*n*	X	S D
374	148 8	5 4	331	160 4	5 9	260	154 9	4 1

[a] Average of height of father and mother

Table 2 *Correlations between heights of parents and their children in rural Guatemalan data*

Age (y)	Sons				Daughters			
	Fathers		Mothers		Fathers		Mothers	
	n	*r*	*n*	*r*	*n*	*r*	*n*	*r*
0 5	186	0 30	251	0 35	155	0 19	202	0 27
1	212	0 37	262	0 33	178	0 22	227	0 22
2	251	0 35	301	0 34	207	0 18	253	0 33
3	253	0 35	299	0 32	235	0 16	275	0 31
4	244	0 33	269	0 30	224	0 24	243	0 40
5	219	0 30	231	0 30	224	0 23	240	0 40
6	199	0 31	201	0 41	206	0 19	211	0 37
7	178	0 24	178	0 42	192	0 17	186	0 38

these populations the hypothesis to be tested is that the correlations obtained in this study will be substantially smaller than those obtained in well nourished populations of European descent

METHODS

The data presented are drawn from an ongoing longitudinal study investigating the effects of chronic malnutrition on mental development and physical growth (9) Four rural ladino communities of Guatemala are being studied These villages are very poor their annual income per family being around $200 (USA) protein calorie malnutrition is endemic and morbidity rates are high

In the study described herein two variables were utilized total body length in children (which will be referred to as height) and standing height in parents Both were measured following standard techniques (22) and adequate measures of quality control (14) Children were examined within ±7 days of the required age Information

Table 3 *Correlations between mid parent heights and heights of their children in the study sample and in USA and Europe*

Age (y)	Sons										Daughters	
	Guatemala		USA (Fels)[a]		USA[b] (Berkeley)		Pool of European cities[c]		Finland[d]		Guatemala	
	n	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*
0 5	182	0 38	170	0 38	32	0 35	141	0 44	–	–	152	0 31
1	202	0 44	175	0 42	29	0 39	139	0 52	55	0 45	172	0 28
2	234	0 42	166	0 45	27	0 36	131	0 49	54	0 46	197	0 34
3	236	0 44	166	0 45	26	0 44	128	0 61	52	0 51	222	0 30
4	219	0 45	162	0 48	25	0 52	134	0 57	54	0 47	200	0 40
5	187	0 45	162	0 48	23	0 50	133	0 58	53	0 43	198	0 38
6	165	0 55	160	0 49	26	0 56	129	0 49	46	0 38	171	0 32
7	145	0 53	153	0 47	24	0 54	129	0 55	42	0 36	150	0 34

[a] Garn (2)
[b] Bayley (1)
[c] Tanner Goldstein & Whitehouse (19)
[d] Tiisala & Kantero (20)

Unfortunately parent–child correlations were not reported in this study

As explained earlier in the introduction on the bases of purely statistical considerations it was expected that parent–child correlations obtained in our study would be substantially smaller in comparison to similar statistics found in studies of European samples As we have seen this is not the case How can this be so? We would like to offer two possible explanations It could be that the magnitude and severity of the environmental insults which the parents suffered as children are positively correlated with those which now plague their own children In other words parents with relatively better nutrition and health are likely to provide a relatively better environment for their children This is suggested by the fact that an index of the family s socioeconomic status a six point scale which summarizes construction materials size and cleanliness of the dwelling (8) is correlated with mid parental height ($r=0\ 3$) in the same magnitude to which it is correlated with the heights of children ($r=0\ 3$) However the partials of parental height on children s heights after controlling for socioeconomic status are not appreciably lower (i e 0 03 to 0 05 less) than the correlations already shown in Tables 3 and 4 Nonetheless the findings are highly suggestive that this explanation partially accounts for the higher than expected correlations observed

An alternative explanation can also be advanced All families of the villages which formed part of the study would be considered poor by most standards The within village variabilities in income health and nutrition are not as large as those to be found in urban settings in developing nations Thus it may be that the variability in environmental conditions is too narrow to noticeably lower the correlations In other words growth is less than maximal in these communities but the extent of growth retardation is more or less constant across individuals In a correlation analysis this would be equivalent to subtracting a constant from both variables the end result being that the original correlation would remain unchanged We have no way of testing this hypothesis within the design of our longitudinal studies

PUBLIC HEALTH IMPLICATIONS

Turning now to the public health use of physical growth as an indicator of nutritional and health status the findings presented are such that one still cannot claim to know how much of the variability in height in malnourished populations is reflective of nutritional and health events In well nourished populations relationships of the same magnitude as those found in our study have served as the basis for proposing parent size specific standards (3 19) Should the use of such standards be proposed for chronically malnourished populations as well? We think that they should not

The use of parent size specific standards would no doubt yield the conclusion that most children in communities similar to the ones studied by us are growing as expected and hence are normal This could be dangerously misleading in that normal for such a setting may be well below optimum Variability in body size among children is related to a series of risks such as morbidity and mortality (16) and to outcomes such as poor mental development Therefore it seems that variability in body size regardless of the relative importance of its causes is a useful public health measure until and unless body size can definitely be ruled out as an indicator of risk Nevertheless the question as to what standards should be used deserves careful consideration More studies such as the one presented here are needed before firm conclusions are reached

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Table 4 *Correlations between siblings heights in USA and Guatemala populations*

Age (y)	Sister–Sister				Brother–Brother				Sister–Brother			
	USA		Guatemala		USA		Guatemala		USA		Guatemala	
	n	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*
0 5	75	0 52	70	0 63	73	0 44	112	0 36	156	0 35	69	0 51
1	74	0 54	98	0 67	74	0 49	132	0 66	154	0 39	95	0 51
2	66	0 62	176	0 64	70	0 50	200	0 52	147	0 39	138	0 50
3	63	0 62	170	0 51	70	0 68	206	0 60	137	0 47	184	0 58
4	61	0 67	108	0 64	63	0 61	182	0 46	123	0 50	174	0 64
5	53	0 73	154	0 52	68	0 57	154	0 37	116	0 54	146	0 54
6	51	0 76	158	0 47	70	0 57	128	0 29	123	0 56	135	0 53
7	52	0 70	144	0 51	67	0 51	114	0 41	114	0 46	107	0 57

Garn & Rohmann (3)

the Berkeley (1) and the Finnish study (20) are higher than the Guatemalan mid parent–daughter correlations in all but two and one instances respectively

Sibling height correlation data are shown in Table 4 for the USA sample (3) and the Guatemalan population studied There appear to be no differences between these two sets of correlations The siblings correlations are higher than the parent–child correlation in the Guatemalan and the USA sample (3) This is not a surprising finding since siblings share a common home environment

DISCUSSION

The results presented in this paper have two types of broad implications The first has to do with current thinking on the nature–nurture debate while the second is concerned with the public health implications of the data We would like to comment on both of these concerns

Nature–nurture debate

Mueller (15) has recently reviewed the literature on parent–child correlation among *school age children* from a variety of genetic and environmental backgrounds He divided the studies into samples of European and non European descent and concluded that father–daughter and mother–daughter correlations for European samples were significantly greater but that this was not the case for father–son and mother–son The absolute differences however do not appear to be very large Moreover because the non European sample is over 50% Japanese and 8 to 9% American Black it is difficult to evaluate the role of chronic malnutrition in this comparison The same author (15) observed that in six samples roughly classified as malnourished height at 7 years of age was positively correlated with father–son and mother–daughter correlations However if the same analyses are carried out for father–daughter and mother–son correlations no relationship is observed

Data on parent–child correlations for pre school children from chronically malnourished populations are scant Some information has been provided for New Guinean populations (11 12 21) but the correlations reported in these studies are difficult to interpret because children of various ages are pooled in the analyses For instance groupings of children 1 to 4 years of age are utilized The statistical noise produced by variability in age would tend therefore to lower the parent–child correlations Pollit & Ricciuti (17) have shown that mothers and fathers of short children are shorter than the parents of relatively taller ones The sample was drawn from children attending day care centers whose working mothers lived in the slums of

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0.5	75	0.52	70	0.63	73	0.44	112	0.36	156	0.35	69	0.51
1	*74*	*0.54*	*98*	*0.67*	*74*	*0.49*	*132*	*0.66*	*154*	*0.39*	*95*	*0.51*
2	66	0.62	126	0.64	70	0.50	200	0.52	147	0.39	138	0.50
3	63	0.62	170	0.51	70	0.68	206	0.60	137	0.47	184	0.58
4	61	0.67	108	0.64	63	0.61	182	0.46	123	0.50	174	0.64
5	53	0.73	154	0.52	68	0.57	154	0.37	116	0.54	146	0.54
6	51	0.76	158	0.47	70	0.57	128	0.29	123	0.56	135	0.53
7	52	0.70	144	0.51	67	0.51	114	0.41	114	0.46	107	0.57

* Garn & Rohmann (3)

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Acta Pædiatr Scand 66 585–587 1977

THE EFFECTIVENESS OF PHENOBARBITAL IN THE PREVENTION OF RECURRENT FEBRILE CONVULSIONS IN CHILDREN WITH AND WITHOUT A HISTORY OF PRE PERI AND POSTNATAL ABNORMALITIES

SHELDON MARK WOLF

From the Department of Neurology Southern California Permanente Medical Group Los Angeles California USA

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KEY WORDS Febrile convulsions phenobarbital

Ouellette in an excellent review of febrile convulsions (FC) in children (6) noted the difficulty in analyzing the prognosis and treatment effect in various series of children with FC because of variations in the proportion of normal children with short generalized seizures in different reports Only Livingston (4 5) she stated had separately presented the date concerning normal children

The purpose of the present study was to evaluate the effects of daily phenobarbital intermittent phenobarbital and no phenobarbital prophylaxis in two groups of children In one group were children with a single brief generalized initial febrile seizure and no history of pre peri or postnatal abnormalities which might suggest the possibility of brain damage and in the other group those with such abnormalities and/or a severe initial febrile convulsion

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PATIENTS AND METHODS

Three hundred and fifty five children with an initial FC seen at the Kaiser Foundation Hospitals in Southern California from 1970 to 1975 were randomized into three groups according to the last digit of the chart number If this was 1–3 (Group I) continuous phenobarbital 3–4 mgm/kgm daily was prescribed with an additional load of 30 mgm/year up to a maximum of 120 mgm at the onset of fever followed by an increase in the dosage to 5 mgm/kgm for the duration of fever If the last digit was 4–6 (Group II) phenobarbital 5 mgm/kgm was prescribed to be given at the onset and for the duration of fever as well as an initial load of 30 mgm/year to a maximum of 120 mgm If the last digit was 7–9 (Group III) no phenobarbital prophylaxis was given If the last digit was 0 the preceding digit was used Fever was defined as a temperature over 100°F The parents of all children in the study were instructed about the importance of quickly lowering the temperature with aspirin (60 mgm/year) and cool water soaks They were also asked to bring the child promptly to the emergency area with any fever so that bacterial infection if present could be treated with antibiotics Subsequent visits were scheduled at 4–6 month intervals Details of compliance and monitoring of blood barbiturate levels are reported elsewhere (10)

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Submitted Nov 2 1976
Accepted Febr 8 1977

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Acta Pædiatr Scand 66 585–587 1977

THE EFFECTIVENESS OF PHENOBARBITAL IN THE PREVENTION OF RECURRENT FEBRILE CONVULSIONS IN CHILDREN WITH AND WITHOUT A HISTORY OF PRE PERI AND POSTNATAL ABNORMALITIES

SHELDON MARK WOLF

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ABSTRACT Wolf Sheldon M (Department of Neurology Southern California Permanente Medical Group Los Angeles California USA) The effectiveness of phenobarbital in the prevention of recurrent febrile convulsions in children with and without a history of pre- peri and postnatal abnormalities Acta Paediatr Scand 66 585 1977.—Three hundred children who had an initial febrile convulsion were divided into two groups In one group were children with a single brief generalized initial febrile seizure and no history of pre- peri or postnatal abnormalities which might suggest the possibility of brain damage and in the other group those with such abnormalities and/or a severe initial febrile convulsion Daily phenobarbital produced a significant decrease in febrile seizure recurrences in both groups as compared to children who received phenobarbital at the onset of fever and no phenobarbital prophylaxis Intermittent phenobarbital given at the onset of fever did not produce a significant difference in recurrence rate as compared with no phenobarbital

KEY WORDS Febrile convulsions phenobarbital

Ouellette in an excellent review of febrile convulsions (FC) in children (6) noted the difficulty in analyzing the prognosis and treatment effect in various series of children with FC because of variations in the proportion of normal children with short generalized seizures in different reports Only Livingston (4 5) she stated had separately presented the date concerning normal children

The purpose of the present study was to evaluate the effects of daily phenobarbital intermittent phenobarbital and no phenobarbital prophylaxis in two groups of children In one group were children with a single brief generalized initial febrile seizure and no history of pre peri or postnatal abnormalities which might suggest the possibility of brain damage and in the other group those with such abnormalities and/or a severe initial febrile convulsion

PATIENTS AND METHODS

Three hundred and fifty five children with an initial FC seen at the Kaiser Foundation Hospitals in Southern California from 1970 to 1975 were randomized into three groups according to the last digit of the chart number If this was 1–3 (Group I) continuous phenobarbital 3–4 mgm/kgm daily was prescribed with an additional load of 30 mgm/year up to a maximum of 120 mgm at the onset of fever followed by an increase in the dosage to 5 mgm/kgm for the duration of fever If the last digit was 4–6 (Group II) phenobarbital 5 mgm/kgm was prescribed to be given at the onset and for the duration of fever as well as an initial load of 30 mgm/year to a maximum of 120 mgm If the last digit was 7–9 (Group III) no phenobarbital prophylaxis was given If the last digit was 0 the preceding digit was used Fever was defined as a temperature over 100°F The parents of all children in the study were instructed about the importance of quickly lowering the temperature with aspirin (60 mgm/year) and cool water soaks They were also asked to bring the child promptly to the emergency area with any fever so that bacterial infection if present could be treated with antibiotics Subsequent visits were scheduled at 4–6 month intervals Details of compliance and monitoring of blood barbiturate levels are reported elsewhere (10)

Children with gross brain damage shigella salmonella

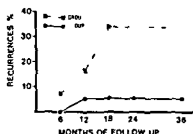


Fig 1 Recurrent FC in children with normal history

or intracranial infection were excluded Continuous phenobarbital was routinely tapered in Group I children at 5½ to 6 years

Life table methodology (1 3) was used to adjust for the different lengths of inclusion in the study The frequency of first febrile seizure recurrences was calculated for all children in the three treatment groups for various intervals following the initial FC The duration of follow up ranged from 6 to 70 months with a mean of 28 months in all groups

For the sake of brevity the two groups will subsequently be called normal and abnormal without meaning to imply that any of the criteria listed below proved that any child actually had brain damage

The abnormal group included children with any one of the following

1 Pregnancy complicated by maternal infection bleeding hypertension toxemia or drug intake (other than vitamins)

Table 1 *Febrile convulsion recurrences in children with normal history*
Group II vs Group III (see text)

Months of follow	Group II (n=31) (%)	Group III (n=23) (%)	p
6	9 7	4 4	NS
12	16 5	18 5	NS
18	26 4	44 9	NS
24	26 4	44 9	NS
36	26 4	44 9	NS

Table 2 *Febrile convulsion recurrences in children with abnormal history*
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Month of follow	Group II (n=87) (%)	Group III (n=67) (%)	p
6	11 6	6 0	NS
12	24 1	20 4	NS
18	28 5	22 4	NS
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36	32 2	32 4	NS

Table 3 *Febrile convulsion recurrences in children with normal history*
Group I vs Groups II+III (see text)

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2 Birth weight under 5 lb
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5 Resuscitation
6 Abnormalities during the neonatal period (as jaundice cyanosis poor suck or cry respiratory difficulty)
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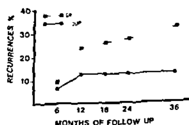


Fig 2 Recurrent FC in children with abnormal history

RESULTS AND CONCLUSION

Of 300 children who could be classified using the above criteria 85 (28%) were in the normal group and 215 (72%) were in the abnormal group In the normal group there were 31 children in Group I 31 children in Group II and 23 in Group III In the abnormal cohort there were 61 in Group I 87 in Group II and 67 in Group III

There were no significant differences in FC recurrence rates between Groups II and III in either the normal or abnormal categories (Tables 1 2) These were combined into one group for further analysis which we call Group II+III

There was a significant difference in recurrence rate between Group I and Group II+III in both categories (Tables 3 4 and Figs 1 2) The difference in recurrence rates in both the normal and abnormal groups was also statistically significant when Group I was compared with Groups II and III separately

These results confirm the studies of others that continuous phenobarbital is effective in preventing recurrent febrile convulsions (2 7 9)

ACKNOWLEDGEMENTS

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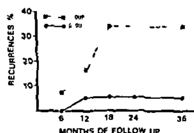


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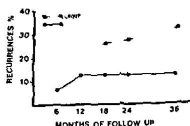


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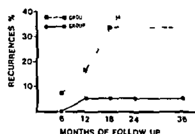


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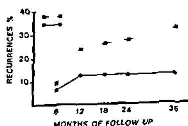


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Acta Pædiatr Scand 66 589–594 1977

SHORT COLON MALFORMATION WITH IMPERFORATE ANUS

AJMER SINGH RAVINDER SINGH and AMARJIT SINGH

From the Department of Surgery and Paediatric Surgery Govt Medical College and Rajindra Hospital Patiala Pb India

ABSTRACT Ajmer Singh Ravinder Singh and Amarjit Singh (Department of Surgery and Paediatric Surgery Govt Medical College and Rajindra Hospital Patiala Pb India) Acta Paediatr Scand 66 589 1977.—Ten cases of a rare high anorectal malformation associated with short colon are presented The significance of a large gas fluid level (occupying more than half of the abdominal cavity on invertogram) is stressed for pre-operative diagnosis Terminal colostomy or ileostomy with disconnection of colo-vesical/colo-vaginal fistula has been considered the only adequate therapy Associated congenital malformations have been responsible for high mortality in these patients

KEY WORDS Short colon colovesical colovaginal colocloacal fistula

Imperforate anus is one of the very common congenital malformations It has a reported incidence of 1 5000 (6) Most of the infants born with this malformation can by and large be categorised by the classification of Stephens & Smith (6) and managed accordingly Associated congenital malformations of various organs are common These render the problem more complex and the management more difficult Association of imperforate anus of high type with a short colon and more often than not a colovesical or colovaginal fistula has been only occasionally reported in the literature This report relates to a series of ten cases encountered at the Rajindra Hospital Patiala Punjab India and a brief review of the available literature The details of these cases are given in Table 1

ILLUSTRATIVE CASE REPORTS

Case 6

K K 84705 a 1-day-old male weighing 700 g was admitted with imperforate anus and history of pneumaturia Plain radiograms in the inverted position vealed a single massive fluid level and air under the abdominal wall indicating pneumoperitoneum (Figs 3*a* and 3*b*) A provisional diagnosis of imperforate anus with short colon colovesical fistula and intestinal performation was made Laparotomy done on the same day revealed free gas in the peritoneal cavity perforation of the caecum absent large gut and a huge distended colonic pouch occupying the entire left half of the abdominal cavity The perforation was closed and a window colostomy performed A biopsy of the colonic wall revealed normal ganglionic pattern Disconnection of the colovesical fistula was not attempted at this stage The child had a good post-operative recovery Intravenous pyelogram done in the postoperative period was normal A cystourethrogram revealed a mild ureteric reflux A contrast study of the colonic pouch performed after introducing a catheter through the colostomy revealed the distended colonic segment and the wide colovesical fistula (Fig 4)

Case 7

K K 91172 a ?-day-old boy weighing ? 500 g was admitted with imperforate anus Invertogram revealed a high type of malformation with a typical large fluid level Laparotomy revealed malrotation of the intestine a massively distended colonic pouch and a colovesical fistula A window colostomy was performed without disconnecting the fistula The child made a quick and uneventful recovery Postoperative cystourethrogram revealed gross ureteric reflux hydroureter and hydronephrosis A contrast study of the colonic pouch done through the colostomy revealed the rudimentary colon and outlined the colovesical fistula (Fig 5)

At a second operation performed 3 weeks after the

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Table I *Showing the details of cases included in the present report*

S no	Age	Sex	Type of malformation and fistula	Associated anomalies	Treatment	Result
1	1 day	M	High colovesical fistula	–	Colostomy	Died
2	3 day	M	High	–	Colostomy	Died
3	1 day	M	High	–	Colostomy	Died 8th day
4	5 day	F	High	–	–	Refused treatment
5	1 day	M	High	Congenital heart disease talipes equino varus	Colostomy	Died 3rd day
6	1 day	M	High colovesical fistula	Perforation of caecum hydro ureter & hydro nephrosis	Colostomy	Survived
7	2 day	M	High colovesical fistula	Malrotation of gut hydroureter and hydronephrosis	Colostomy and closure of fistula	Survived
8	2 day	M	High fistula?	Malrotation of gut	Colostomy	Died 6th day
9	7 mth	M	High colovesical fistula	Malrotation of gut	Colostomy closure fistula abdomino-perineal pull through	Survived now 6 yrs old
10	20 day	F	High colocloacal fistula	Double uterus malrotation of gut Meckel s diverticulum and absent appendix	Colostomy and closure of fistula	Died 18th day

first the colovesical fistula was disconnected The child recovered well and was sent home with a well functioning colostomy and is under follow up

Case 9

N S 97354 a 6 year old boy was first seen at 7 months of age with a history of repeated obstruction of colostomy The child had been born with apparently a high type of anal imperforation for which an unsuccessful perineal exploration and a caecostomy had been done elsewhere Laparotomy at that time revealed a short colon and malrotation of the intestine The caecostomy was refashioned at this stage At a subsequent operation done at 13 months of age a colovesical fistula was disconnected and an abdominoperineal pull through done The child recovered well

At 6 years of age the child now has considerable abdominal distension with complete fecal incontinence Barium meal and enema series (Fig 6 *a*) revealed a massively distended colonic pouch occupying more than the left half of abdomen

A cystourethrogram (Fig 6 *b*) demonstrated gross displacement of the urinary bladder to the right side

Case 10

B G 95667 a 20-day old female weighing 3 000 g was admitted with congenital absence of anus and history of passage of stools per vaginam Examination revealed a female configuration of external genitalia with a single opening through which the baby was passing both urine and stools The anus was absent at the normal site Plain invertograms showed a large fluid level Contrast studies through the perineal opening revealed a large intra abdominal cavity A clinical diagnosis of cloaca with short colon and colo-cloacal fistula was made Exploration of the abdomen revealed a large colonic pouch communicating below with the upper and posterior part of the cloaca which in turn was communicating further inferiorly but on its anterior surface with the urinary bladder There was a double uterus opening into the cloaca from either side A Meckel s diverticulum and intestinal malrotation were also present The colo-cloacal fistula was disconnected and a window colostomy done A biopsy of the colon sent for histological examination revealed normal ganglion cells The patient had an exceptionally good postoperative recovery but died after aspirating a feed on the eighteenth postoperative day Autopsy confirmed the operative findings There was no associated anomaly

OBSERVATIONS

In this series eight infants were males and two were females All had the high supralevator type of anorectal malformation Nine of these cases were operated upon In all of them on opening the abdomen the terminal ileum was found to enter a thin massively distended co

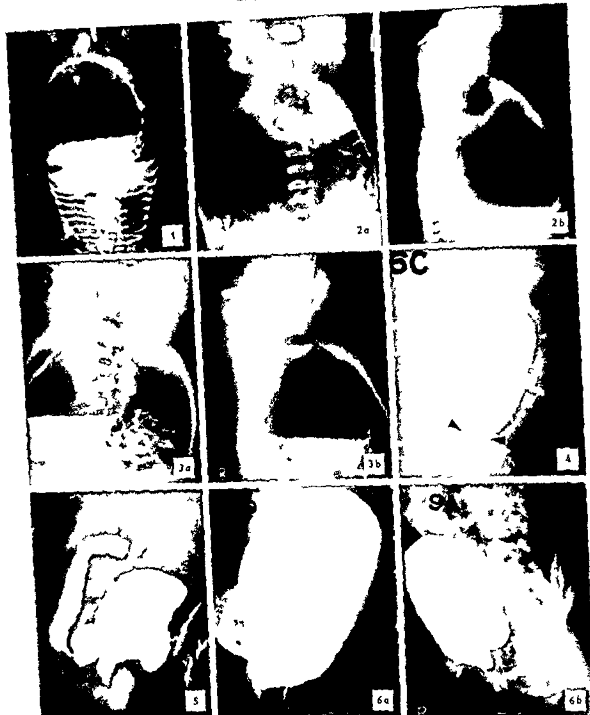


Fig 1 Invertogram of case 2 showing the typical fluid level *bigger than* half the abdominal capacity

Fig 2 a Invertogram of case 5 showing the typical fluid level the colovesical fistula and the gas filled urinary bladder

Fig 2 b Lateral invertogram of case 5 showing the large colonic fluid level and gas in the urinary bladder

Fig 3 a Invertogram of case 6 showing the short *colon fluid level* pneumoperitoneum and gas in the bladder

Fig 3 b Lateral invertogram of *case 6 showing* the large fluid level pneumoperitoneum and *gas in the bladder*

Fig 4 Cologram of case 6 showing the colovesical fistula

Fig 5 Cologram of *case* 7 *showing* the colonic pouch and the colovesical fistula *The terminal* ileum has also been outlined and its low *entry into* the colonic pouch visualised

Fig 6 a Barium enema of case 9 *showing* the massively distended colonic pouch

Fig 6 b Cystourethrogram of case 9 showing a right-sided displacement of urinary bladder

Table 1 *Showing the details of cases included in the present report*

S no	Age	Sex	Type of malformation and fistula	Associated anomalies	Treatment	Result
1	1 day	M	High colovesical fistula	–	Colostomy	Died
2	3 day	M	High	–	Colostomy	Died
3	1 day	M	High	–	Colostomy	Died 8th day
4	5 day	F	High	–	–	Refused treatment
5	1 day	M	High	Congenital heart disease talipes equino varus	Colostomy	Died 3rd day
6	1 day	M	High colovesical fistula	Perforation of caecum hydro ureter & hydro nephrosis	Colostomy	Survived
7	2 day	M	High colovesical fistula	Malrotation of gut hydroureter and hydronephrosis	Colostomy and closure of fistula	Survived
8	2 day	M	High fistula?	Malrotation of gut	Colostomy	Died 6th day
9	7 mth	M	High colovesical fistula	Malrotation of gut	Colostomy closure fistula abdomino-perineal pull through	Survived now 6 yrs old
10	20 day	F	High colocloacal fistula	Double uterus malrotation of gut Meckel s diverticulum and absent appendix	Colostomy and closure of fistula	Died 18th day

first the colovesical fistula was disconnected The child recovered well and was sent home with a well functioning colostomy and is under follow up

Case 9

N S 97354 a 6 year-old boy was first seen at 7 months of age with a history of repeated obstruction of colostomy The child had been born with apparently a high type of anal imperforation for which an unsuccessful perineal exploration and a caecostomy had been done elsewhere Laparotomy at that time revealed a short colon and malrotation of the intestine The caecostomy was refashioned at this stage At a subsequent operation done at 13 months of age a colovesical fistula was disconnected and an abdominoperineal pull through done The child recovered well

At 6 years of age the child now has considerable abdominal distension with complete fecal incontinence Barium meal and enema series (Fig 6 *a*) revealed a massively distended colonic pouch occupying more than the left half of abdomen

A cystourethrogram (Fig 6 *b*) demonstrated gross displacement of the urinary bladder to the right side

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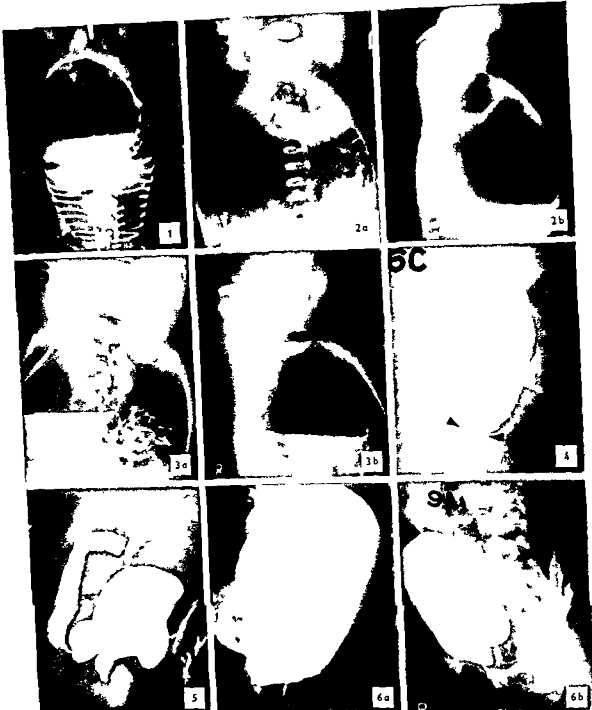


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Fig 6 b Cystourethrogram of case 9 showing a right sided displacement of urinary bladder

Table 2 *Showing the details of cases reported in literature*

Author	S no	Age	Sex	Type of malformation and fistula	Associated anomalies
Trusler et al (7)	1	1 day	M	High colovesical fistula	Hydronephrosis hypospadias and undescended testis
	2	1 day	F	High colovaginal fistula	Double appendix
	3	1 day	F	High vesicovaginal fistula	Malrotation of gut hydroureter hydronephrosis bicornuate uterus
	4	1 day	M	High	Double appendix hydroureter hydronephrosis undescended testis hypospadias hemiverte brae talipes equino-varus
	5	1 day	M	High	–
	6	1 day	F	High colovaginal fistula	Double uterus double vagina
	7	1 day	F	High colovesical fistula	Absent appendix bicornuate uterus
Spriggs (5) *as quoted by* Trusler	1	Museum *specimen*		–	–
Dickinson (2)	1	1 day	M	High	Double appendix duplication cyst
Blunt & Rich (1)	1	1 day	F	High	Left renal aplasia absent left ovary
Shafie (3)	1	3 day	F	Low ectopic perineal anus	–
Singh & Pathak (4)	1	8 day	M	High colovesical fistula	Absent appendix
	2	45 day	F	High colovaginal fistula	Malrotation of gut
	3	4 day	M	High colovesical fistula	Malrotation of gut Ladd s band double appendix duplication cyst
	4	3 day	M	High colovesical fistula	–
	5	1 day	M	High colovesical fistula	–
	6	3 day	M	High colovesical fistula	Absent caecum and appendix hydrenephrosis

lonic pouch All attempts to deliver this pouch outside the wound for a loop colostomy failed and invariably a side or window colostomy had to be performed except in one case in which a catheter drainage of the sac was done

Associated malformations were very com mon in the cases included in this series Malro tation of the intestine was seen in four hydroureter and hydronephrosis in two dou ble uterus with a cloacal outlet in one and congenital heart disease in another case In case 6 there was perforation of the caecum with free air in the peritoneal cavity

Five of the operated cases (Nos 1 2 3 5 and 8) died within a few days In these cases a colovesical or colovaginal fistula could not be demonstrated However the invertograms of the first case clearly revealed a colovesical fistula by the presence of gas in the uri nary bladder In the remaining four cases detailed investigations such as colograms cystourethrograms excretory pyelograms barium studies colonic biopsies and re exploration of the abdomen were carried out In all these cases there was either a patent colovesical or a colocloacal fistula The fistu lous communication was always found to be very wide with easy and free flow of the con trast from the colonic pouch to the urinary bladder Another interesting finding was that the fistula opened into the bladder high up on its posterior surface below the fundus This is

Treatment	Result
-	Died 24 hours after birth
Colostomy	Survived
Colostomy	Died 3rd day
Colostomy	Died
Closure fistula colostomy	Survived
Closure fistula ileostomy	Died
Colostomy closure fistula	Survived
-	-
Ileostomy	Died
Caecostomy	Died 2nd day
Perineal anoplasty—failed colostomy	-
Ileostomy	Died
Colostomy closure fistula	Survived
-	Died 1 hr after birth
Colostomy	Survived
Colostomy closure fistula	Survived
Closure fistula colostomy	Died

in contrast to the usual finding in high anorec tal malformations where the *hind gut* com municates with the bladder just above the *in* ternal meatus

Another observation worth considering was that the terminal ileum opened into the short colonic pouch inferiorly very close to the site of the fistula This low entry of the ileum into the colonic pouch renders proper mobilisation of the colon for a definite pull through proce dure impossible In two cases biopsies taken from the colon wall showed normal ganglionic cells In the last case the superior mesenteric artery and its branches were found to supply the colonic pouch while the inferior mesen teric artery was missing

DISCUSSION

Trusler et al in 1959 (7) were probably the first to report in a series of seven cases the oc currence of short colon malformation in as sociation with imperforate anus They re viewed the literature and referred to a similar report by Spriggs (5) of a museum specimen consisting of imperforate anus and absent left colon Similar case reports have been made by Dickinson (2) Blunt & Rich (1) Singh & Pa thak (4) and Shafie (3) making a total of seven teen cases so far reported in the literature These cases are reviewed in Table 2

Although anorectal malformations are one of the commonest anomalies of the newborn (1 5000) it is very strange that so far only 11 cases have been reported having an associated short colon malformation from outside India Singh & Pathak (4) in 1972 reported six cases from Chandigarh India It is interesting to note that Chandigarh is only 70 kilometers from Patiala and both these places contain similar Punjabi populations How is it that this malformation is so common in this region? In our hospital short colon was encountered in roughly 4 38% of all cases of anorectal malformations and constituted approximately 10% of all cases with high malformation

We first became aware of this malformation in 1970 when the senior author operated upon case 9 The reports of Trusler et al (7) and Singh & Pathak (4) reinforced our awareness Since then a correct preoperative diagnosis has been possible in all the cases We agree with Singh & Pathak (4) that the most characteristic feature of short colon malforma tion which permits a correct preoperative diagnosis is the large fluid level obtained on an invertogram occupying nearly half and often more than half the abdominal cavity (Figs 1 2*a* and 2*b*)

On exploration of the cases one finds a thin congested distended sac representing the co lon The delivery of this sac for a loop co lostomy is impossible In our opinion the oc currence of a colovesical or a colovaginal fistula is more or less a standard feature of

short colon It was present in six of the seven cases reported by Trusler (7) and in all cases reported by Singh & Pathak (4) It was encountered in five of our more thoroughly investigated cases Pneumaturia in an infant indicates the presence of a colovesical fistula Invertograms may show gas in the bladder and often may also outline the gas filled fistula (Fig 2*a*) We have already emphasised the unusually large calibre of the fistula and its high opening into the bladder in our cases

Although male preponderance (8 2) is very striking in our cases as well as in the Singh & Pathak (4) series (5 1) it was not observed in other reports Short colon was associated with high anorectal malformation in 26 out of 27 cases so far reported The atypical case of Shafie (3) had short colon associated with perineal ectopic anus A high incidence of associated congenital malformations of other systems such as the gastrointestinal genitourinary cardiovascular etc has been mentioned in all reports

As regards management we disagree with the suggestion of Trusler (7) of an abdominoperineal pull through in short colon malformation We feel that this would fail because of a low entry of the terminal ileum into the colonic pouch and tailoring of the distended colon does not result in a proper evacuation of the colon We recommend a terminal colostomy after excision of the redundant colon or a terminal ileostomy as the most desirable procedure after disconnection of the fistula

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Submitted Aug 30 1976
Accepted Oct 26 1976

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Acta Pædiatr Scand 66 595–600 1977

ECHOCARDIOGRAPHIC ASSESSMENT OF LEFT VENTRICULAR FUNCTION DURING THE INJECTION OF ADRIAMYCIN

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ABSTRACT Bjorkhem G and Garwicz S (Department of Paediatrics University Hospital Lund Sweden) Echocardiographic assessment of left ventricular function during the injection of adriamycin Acta Paediatr Scand 66 595 1977 —Echocardiography was used to evaluate left ventricular function in 8 children treated with adriamycin for malignant disease Preejection period (PEP) left ventricular ejection time (LVET) and percent change in left ventricular internal dimension with systole (ΔLVID) were measured before during and immediately after 22 injections of adriamycin as well as 14 injections of other cytotoxic drugs and physiologic saline No immediate effects on left ventricular function could be discerned When functional parameters were evaluated longitudinally in patients with relatively higher cumulative doses of adriamycin percent change in left ventricular internal dimension with systole showed some tendency to decrease while the other parameters remained essentially unchanged

KEY WORDS Echocardiography adriamycin cardiotoxicity

Antibiotics of the anthracycline group particularly adriamycin have been shown to possess a significant activity against several malignancies and are therefore widely used (1) It appeared quite early that some patients treated with these drugs developed unexplained and often refractory heart failure Subsequent reports established the fact that the incidence of cardiomyopathy increased at higher cumulative doses of adriamycin and that a total dose of up to 500 mg/m² body surface is safe in terms of cardiac toxicity (4 8) However this may not be valid in patients who have received irradiation to the thorax which appears to potentiate the cardiotoxicity of adriamycin (3 4) The possibility of interaction with other drugs in this respect cannot be excluded either Moreover a recent study (11) showed that left ventricular function is impaired already after adriamycin in doses of 310–540 mg/m² Transient decrease in function occurred even in patients whose cumulative dose was less than 200 mg/m when these were studied 2 weeks after therapy by 4 weeks after therapy the left ventricular function returned to normal (11) Because the distribution of adriamycin after intravenous injection shows a rapid uptake in cardiac tissue (16) it seemed of interest to investigate left ventricular function during and immediately after the administration of the drug As a most appropriate method of studying this function echocardiography was chosen as reported below

PATIENTS AND METHODS

Eight children receiving adriamycin (Adriablastina Farmitalia) for the treatment of acute myeloblastic leukaemia (4 patients) acute lymphoblastic leukaemia in relapse (? patients) osteogenic sarcoma (1 patient) and leukaemic phase of lymphoblastic lymphoma (1 patient) were studied All of them were in good general condition and none showed signs of heart disease judged by history physical examination electrocardiography (ECG) and

short colon It was present in six of the seven cases reported by Trusler (7) and in all cases reported by Singh & Pathak (4) It was encountered in five of our more thoroughly investigated cases Pneumaturia in an infant indicates the presence of a colovesical fistula Invertograms may show gas in the bladder and often may also outline the gas filled fistula (Fig 2*a*) We have already emphasised the unusually large calibre of the fistula and its high opening into the bladder in our cases

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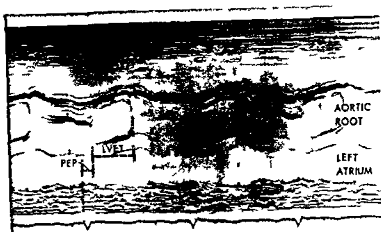


Fig 1 Echocardiogram of the aortic root showing PEP and LVET measurements

90 and 110% (unpublished material) PEP/LVET is claimed not to be influenced by heart rate or age and normal mean value is 0 313±0 05 (S D) (13)

Left ventricular internal dimensions were measured close to the tip of the anterior mitral leaflet where clear echoes from the endocardium and the left side of the septum could be obtained (Fig 2) The end-diastolic left ventricular internal dimension ($LVID_d$) was measured at the beginning of the QRS-complex and the end systolic left ventricular internal dimension ($LVID_s$) as the shortest distance between the septum and the endocardium in end systole ΔLVID was then calculated as

$$\frac{LVID_d - LVID_s}{LVID_d}$$

$LVID_d$ and $LVID_s$ were calculated as the mean of several consecutive beats and ΔLVID was compared to the normal mean value 36±4% (S D) given by Gutgesell et al (5) It was often not possible to obtain clear echoes for measuring PEP LVET and ΔLVID at each registration but when five or more measurements could be made at appropriate intervals during and after the injection a curve for each parameter was constructed to show the changes with time

RESULTS

The curves reflecting the changes with time for LVET % PEP/LVET and ΔLVID before during and after the injection of adriamycin were quite conformant showing only moderate variations and usually within the normal limits for the parameter (Fig 3) No certain

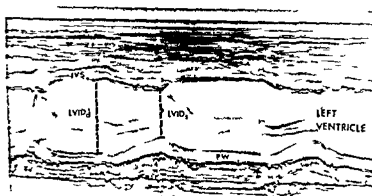


Fig 2 Echocardiogram of the left ventricle showing sites for measuring $LVID_d$ and $LVID_s$ *IVS* interventricular septum *PW* posterior wall of the left ventricle

Table 1 *Clinical data and parameters of left ventricular function in patients treated with adriamycin*

ALL=acute lymphoblastic leukaemia AML=acute myeloblastic leukaemia CNS=central nervous system a=vincristine sulfate b=methotrexate c=6 mercaptopurine d=cyclophosphamide e=cytosine arabinoside g=thioguanine

Pat	Sex Age (y)	Diagnosis	Stage of the disease	Investigation no	Cumulative dosis of adriamycin before injection (mg/m²)	Other cytotoxic drugs used previously	Echocardiographic parameters before injection ΔLVID (%)	LVET (% of normal)	PEP/LVET
A	M 12	ALL	Third relapse	1	270	a b c d e	34	97	0 36
				2	300	a b c d e	26	95	0 33
B	M 8	Osteogenic sarcoma	Remission	1	240	d	36	110	0 35
				2	270	d	43	101	0 33
				3	420	d	37	111	0 25
				4	450	d	29	103	0 23
C	M 13	AML+CNS involv	Remission	1	0	a b e g	16	92	0 48
				2	120	a b e g	24	108	0 38
				3	160	a b e g	23	98	0 38
				4	200	a b e g	21	93	0 36
				5	240	a b e g	23	100	0 28
D	M 8	AML	First blastic phase	1	0	a b	33	92	0 35
				2	20	a b	34	94	0 39
				3	60	a b	33	96	0 31
E	M 12	ALL	First relapse	1	0	a b c d	33	104	0 23
				2	20	a b c d	32	108	0 24
F	F 9	AML	Remission	1	160	a b e g	32[a]	100[b]	0 24[b]
				2	180	a b e g	37	106	0 24
G	F 5	AML	First blastic phase	1	0	a	32	101	0 24
				2	20	a	32	98	0 24
H	M 7	Lymphoma	Leukaemic phase	1	0	a b c d e	37	97	0 19
				2	20	a b c d e	50	92	0 23

[a] Value obtained at the beginning of the injection
[b] Value obtained after the injection

chest X ray All patients were previously treated with other cytotoxic agents (Table 1) Adriamycin was used alone in doses of 30–40 mg/m² in patients A B and C in a dose of 20 mg/m² in combination with vincristine in patients D E G and H and in the same dose in combination with cytosine arabinoside in patient F Thirty six echocardiographic recordings were obtained during 22 injections of adriamycin 8 injections of vincristine 2 injections of cytosine arabinoside 1 injection of cyclophosphamide and 3 injections of physiologic saline Adriamycin was given as a rapid intravenous injection during 1 to 2 minutes after dilution in sterile water to a concentration of 2 mg/ml

The echocardiograms were recorded with an Echocardiovisor ultrasonoscope (Organon Technica) with a fiber optic recorder using a 4 5 MHz unfocused transducer Examinations of the aortic leaflets and the left ventricle were made before the injection was started during the injection and then at about one minute intervals during the following ten minutes The transducer was held in standard positions (2) and care was taken to obtain the same position for registering the left ventricle each time During a few examinations a constant transducer position was ensured by registering only the left ventricle during and after the injection The degree of variation in left ventricular internal dimension in this situation was comparable to that found when the position of the transducer was changed between left ventricle and aortic root Registrations from the aortic leaflets were made using a paper speed of 100 mm/sec and from the left ventricle with a paper speed of 50 or 100 mm/sec

From the echocardiograms left ventricular preejection period (PEP) left ventricular ejection time (LVET) and left ventricular internal dimensions in end diastole and end systole ($LVID_d$ $LVID_s$) were measured PEP was measured from the beginning of the QRS complex to the opening of the aortic leaflets and LVET from the opening to the closing of the aortic leaflets (Fig 1)

LVET was corrected for heart rate using the regression equation of Spitaels et al (13) and expressed as LVET percent of normal (LVET %) From PEP and LVET the quotient PEP/LVET was also calculated In left ventricular dysfunction LVET % decreases and PEP/LVET increases (15) Excluding the newborn period LVET is not influenced by age and can be corrected for heart rate to LVET % (6 13) The normal range lies between

tracing and phonocardiogram (15) but especially in children echocardiography is an easier way of obtaining these parameters and an excellent correlation between values measured from echoes and from carotid pulse tracings has been shown (7)

In several studies PEP/LVET and ΔLVID have been shown to be the most sensitive measures of left ventricular dysfunction (5 14) and therefore these parameters were chosen in this study also including LVET % as another parameter independent of heart rate and age

Rinehart et al have recently shown that chronic adriamycin induced cardiac injury can be detected from systolic time intervals before clinical signs appear (11) Since adriamycin reaches high levels in cardiac muscle very rapidly after an intravenous injection (16) and transient ECG changes sometimes can be seen (4 8) this study was undertaken to study the immediate effects of adriamycin on left ventricular function No significant alteration in left ventricular function could be found as judged by PEP/LVET LVET % and ΔLVID during and immediately after the injection of adriamycin With the cumulative doses of adriamycin reached in this study the functional parameters also remained essentially stable The two patients A and B who received the highest cumulative doses showed only a slight decrease in ΔLVID These findings are difficult to evaluate yet but have to be followed up with further longitudinal studies

Patient C differs from the rest of the group His left ventricular function was impaired before the first injection of adriamycin and actually improved during treatment We have no ready explanation to this but it is possible that he had primary myocardial engagement of the malignant disease that improved with treatment

In conclusion no immediate negative effect of adriamycin on left ventricular function could be shown but a careful follow up of patients receiving this drug is necessary Echocardiography makes it possible to study left ventricular function regularly in these patients detect early changes and alter treatment accordingly

ACKNOWLEDGEMENT

Supported by grants from the Swedish National Association against Heart and Chest Diseases

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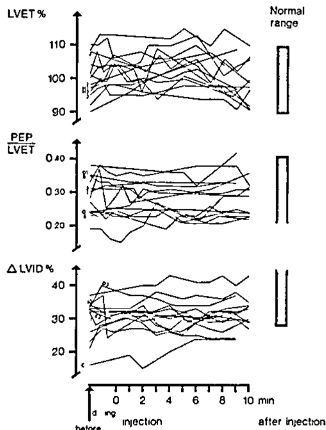


Fig 3 Parameters of left ventricular function (LVET PEP/LVET ΔLVID) before during and after the injection of adriamycin Normal range for each parameter is indicated Letters and numbers signify patients and investigation no as used in Table 1

trend for the variations could be discerned The same was found when other cytotoxic agents as vincristine cyclophosphamide and cytosine arabinoside were injected and also during the injection of physiologic saline Fig 4 shows the similarities of the curves when adriamycin vincristine and physiologic saline were injected in the same patient Left ventricular function was also followed longitudinally by comparing functional parameters at the beginning of each examination (Table 1) Patient A and B reached a cumulative dose of adriamycin of 300 and 450 mg/m² respectively during the study period and showed a slight decrease in ΔLVID during the same period but so far normal LVET % and PEP/LVET Patient C showed clearly pathological values before the start of this investigation and without previous treatment with adriamycin His initial ΔLVID was 16% and PEP/LVET 0 48 Left ventricular function improved in this patient during treatment with adriamycin reaching normal values for PEP/LVET and improving but still abnormal ΔLVID

DISCUSSION

Echocardiography offers an excellent tool in heart investigation in both adults and children (2 9) The method is direct yet non invasive and can be used to assess left ventricular function both from left ventricular contractility (5 10 12) and from systolic time intervals (7) Systolic time intervals have earlier mostly been derived from ECG indirect carotid pulse

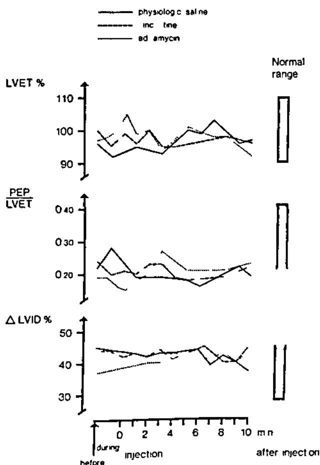


Fig 4 Parameters of left ventricular function (LVET PEP/LVET ΔLVID) before during and after consecutive injections of vincristine physiologic saline and adriamycin in the same patient

tracing and phonocardiogram (15) but especially in children echocardiography is an easier way of obtaining these parameters and an excellent correlation between values measured from echoes and from carotid pulse tracings has been shown (7)

In several studies PEP/LVET and ΔLVID have been shown to be the most sensitive measures of left ventricular dysfunction (5 14) and therefore these parameters were chosen in this study also including LVET % as another parameter independent of heart rate and age

Rinehart et al have recently shown that chronic adriamycin induced cardiac injury can be detected from systolic time intervals before clinical signs appear (11) Since adriamycin reaches high levels in cardiac muscle very rapidly after an intravenous injection (16) and transient ECG changes sometimes can be seen (4 8) this study was undertaken to study the immediate effects of adriamycin on left ventricular function No significant alteration in left ventricular function could be found as judged by PEP/LVET LVET % and ΔLVID during and immediately after the injection of adriamycin With the cumulative doses of adriamycin reached in this study the functional parameters also remained essentially stable The two patients A and B who received the highest cumulative doses showed only a slight decrease in ΔLVID These findings are difficult to evaluate yet but have to be followed up with further longitudinal studies

Patient C differs from the rest of the group His left ventricular function was impaired before the first injection of adriamycin and actually improved during treatment We have no ready explanation to this but it is possible that he had primary myocardial engagement of the malignant disease that improved with treatment

In conclusion no immediate negative effect of adriamycin on left ventricular function could be shown but a careful follow up of patients receiving this drug is necessary Echocardiography makes it possible to study left ventricular function regularly in these patients detect early changes and alter treatment accordingly

ACKNOWLEDGEMENT

Supported by grants from the Swedish National Association against Heart and Chest Diseases

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Submitted Jan 22 1977
Accepted Febr 26 1977

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Acta Pædiatr Scand 66 601–604 1977

STUDIES ON MATURITY IN NEWBORN INFANTS

IX *Further Observations on the Use of External Characteristics in Estimating Gestational Age*

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ABSTRACT Finnstrom O (Department of Paediatrics University Hospital Linkoping Sweden) Studies on maturity in newborn infants IX Further observations on the use of external characteristics in estimating gestational age Acta Paediatr Scand 66 601 1977 — A simple method for assessing maturity based on 8 external characteristics has previously been described Some new results with the use of this method are now presented The precision in estimating gestational age is sufficiently good more than 95% of the infants were correctly estimated within ±3 weeks Reducing the criteria to seven and thereby excluding the ophthalmoscopic examination did not significantly change the precision of the estimation The examination based on external characteristics is a very simple and useful method for maturity assessment even in sick newborn infants Tables for transforming maturity score into gestational age are presented

KEY WORDS Maturity gestational age newborn infants external characteristics

In a series of earlier papers the author discussed and compared several methods for assessing maturity in newborn infants (2 7–13) In these studies the scoring of external characteristics was recommended as the method of choice for routine work (11 12 13) The method has been used with highly satisfactorily results in the routine clinical work in our Department In the original description 8 external characteristics were included The use of pupillary membranes necessitating an ophthalmoscopic examination has been of some concern to several clinicians however We therefore thought it could be of value to assess the method comparing the estimation of gestational age using either 8 or 7 criteria i e excluding the eye examination We also want to give some more information regarding the accuracy in determining gestational age by this method and discuss the problem of maturity assessment in the light of the recent literature The examination technique will be presented in some detail again and also simple tables for transforming maturity score into gestational age

MATERIAL

Criteria for selection

Only infants for whom reliable information about gestational age was available were included in the study The criteria have been given in detail previously (7)

Definitions and abbreviations used

Gestational age age in days from the first day of the mother s last menstrual period until the day of birth ***40th week*** days 274 to 280 ***SGA*** small for gestational age Infant with birth weight below −2 S D in the relation between birth weight and gestational age according to Swedish standard curves ***AGA*** appropriate for gestational age Infant with birth weight within normal limits for the gestational age (between −2 and +2 S D) ***LGA*** large for gestational age Infant with birth weight above +2 S D according to Swedish standard curves ***Preterm*** gestational age less than 267 days postmenstrual ***Term*** gestational age between 267 and 294 days ***Postterm*** gestational age more than 294 days postmenstrual ***Maturity*** as used by the authors maturity is an expression of the degree of development of the newborn infant Maturity thus defined is dependent on the gestational age of the infant but also on other factors such as biological variation ***LMP*** last menstrual period

Table 1 *Transforming maturity score to gestational age (days)*

Maturity score (8 criteria)	Gestational age (days)	Maturity score (7 criteria eyes excluded)	Gestational age (days)
8	192	7	191
9	197	8	198
10	203	9	204
11	209	10	211
12	215	11	217
13	220	12	224
14	226	13	230
15	232	14	237
16	237	15	243
17	243	16	250
18	249	17	256
19	255	18	263
20	261	19	269
21	266	20	276
22	272	21	282
23	278	22	289
24	284	23	295
25	289		
26	295		

Investigated material

Details of this material have been given in an earlier paper (12) The distribution in different gestational age groups was as follows Below 225 days (5 infants) 225–238 (10) 239–252 (13) 253–266 (29) 267–280 (35) above 280 (33) Total number of infants 125 Only 2 infants were SGA 2 were LGA

METHODS

The infants were examined on the first or second day of life by one examiner Eight criteria were examined and scored according to 2 to 4 alternative responses (scores) as follows

1 *Breast size* The transverse diameter is measured bi laterally with a sliding caliper and the largest value re gistered (*a*) below 5 mm (*b*) 5 to 10 mm (*c*) more than 10 mm

2 *Nipple formation* Estimated by inspection (*a*) nipple barely visible no areola (*b*) nipple well defined areola present but not raised (*c*) nipple well defined edge of the areola raised above the skin

3 *Skin opacity* Estimated by inspection of the trunk (*a*) numerous veins tributaries and venules are clearly seen particularly over the abdomen (*b*) veins and tri butaries are seen (*c*) a few large blood vessels are clearly seen over the abdomen (*d*) a few large blood vessels are seen indistinctly over the abdomen or no blood vessels are seen

4 *Scalp hair* The scalp hair is inspected (*a*) fine hair wooly or fuzzy individual strains difficult to dis tinguish (*b*) hair coarse and silky Each hair appears as a single strand

5 *Ear cartilage* The ears are palpated in order to estimate the distribution of ear cartilage In case there is a difference between the two ears the judgment is based on the most mature ear (*a*) no cartilage is felt in anti tragus (*b*) cartilage is felt in antitragus (*c*) cartilage is present in anthelix (*d*) cartilage formation is completed in helix (i e cartilage can be palpated in the dorsal cranial part)

6 *Finger nails* The finger nails are inspected and the finger tip palpated (let the nail scratch the hand of the examiner) (*a*) the nails do not reach the fingertips (*b*) the nails reach the fingertips (*c*) the nails reach or pass the finger tips distal edge of the nail is distinct and relatively firm (i e the edge of the nail can easily be felt if the nail scratches the hand of the examiner)

7 *Plantar skin creases* The sole of the foot is in spected Only the relatively broad creases are analysed Fine superficial lines may be present especially if the skin is dry but usually disappear if the sole is stretched from toes to heel (*a*) no skin creases are present (*b*) anterior transverse creases only are present (*c*) occasional creases are seen on the anterior two-thirds of the sole (*d*) the whole sole is covered with creases i e also the heel

8 *Pupillary membranes* The pupillary plane is examined after widening of the pupils with a short acting mydriatic agent with the aid of an ophthalmoscope (*a*) membrane rests are seen in the form of distinct arcades (*b*) one or several separate strands are seen uni or bi laterally (*c*) no membrane rests are seen over the widened pupil

Criteria number 2 and 3 are essentially as described by Farr et al (5) number 4 and 7 according to Usher et al (25) and number 5 6 and 8 essentially after von Harnack & Oster (15)

Gestational age can be calculated as follows The individual scores are summed this sum (total maturity score) is divided by the maximal maturity score possible for that infant (depending on how many criteria that are used) and estimated gestational age is calculated from the formula Y(days)=145 62+149 38 X(8) More simply the total maturity score for 8 or 7 criteria can be transformed to gestational age in days according to Table 1

RESULTS

The results of the calculations of gestational age from maturity scores are given in Table 2 in comparison with gestational ages based upon LMP Thus a correct estimation of gestational age within ±2 weeks was obtained in 88% of the cases 98% of the cases were correctly estimated within ±3 weeks There were practically no differences in the results using 7 or 8 criteria in calculating gestational age For short gestational ages there is a tendency to overestimate the gestational ages There was also a slight tendency to under estimate gestational age after post term de liveries

Table 2 *Number of infants correctly estimated within ±2 and 3 weeks respectively*

	Correct estimation within ±2 weeks (14 days)		Correct estimation within ±3 weeks (21 days)	
	8 criteria	7 criteria	8 criteria	7 criteria
All infants (*n*=125)	110 (88%)	109 (87 2%)	123 (98 4%)	122 (97 6%)
Gestational age <267 days (*n*=57)	49	48	57	56
Gestational age 267–294 days (*n*=59)	55	55	58	58
Gestational age >294 days (*n*=9)	6	6	8	8

DISCUSSION

The present investigation further support the practical value of this method based on external characteristics for maturity assessment in the newborn period The examination is sufficiently exact also at low gestational ages It is less suitable in differentiating between term and post term pregnancies Practically all infants were correctly estimated regarding age to within ±3 weeks which corresponds well with the theoretically estimated precision (11) The few cases in which the gestational age was miscalculated might well depend on incorrect figures for LMP given by the mother although these figures were carefully controlled Several other authors also showed the value of external characteristics in assessing gestational age (1 4 6 17 22 24)

Some previous authors claim that it is possible to estimate gestational age to within 1–2 weeks These statements were based on the calculated 95% confidence limits for estimating gestational age at mean values of the scores However as pointed out previously (11) general conclusions cannot be drawn from these confidence limits since they have been constructed from materials that differ very much regarding the distribution of gestational ages Meyer Dietrich (20) in a recent study on mainly pre term infants using the Farr (5) and Dubowitz (4) scoring system got considerably broader confidence limits as did Brueton et al in an earlier study (3) He also pointed out that differences in confidence limits depend more on differences in the composition of the materials than on differences in the work up of the studies or the quality of the tests used

In an earlier paper we said that the precision in estimating gestational age was less when the number of criteria was reduced from 8 to 7 in our method (8) From a practical point of view as showed in this paper this difference is too small to be of any importance Thus it is not necessary to use the ophthalmoscopic examination which some clinicians find difficult to perform

In a similar manner Nicolopoulos et al (21) recently showed that it is possible to reduce the number of criteria in the Farr score from 11 to 9 and the number of criteria in the neurological part of the Dubowitz score from 10 to 8 without losing precision in the estimation of gestational age

Parkin et al (23) who also used the Farr score even suggested that it would be possible to reduce the number of criteria in this examination to only 4 However at low values of the score a change in only one point of the total score leads to much greater changes in gestational age than at higher values of the score The method based on only four criteria seems to be rather inexact for low scores There is probably therefore an optimal number of items which should not be reduced (in our scoring this is 7 criteria)

Dubowitz et al (4) showed earlier that the precision in estimating gestational age was increased when a combination of external characteristics and neurological tests were used as did the present author (11) This finding has been confirmed by others (21) but has

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Acta Pædiatr Scand 66 605–610 1977

TREATMENT OF SEPTICAEMIA IN THE NEWBORN INFANT CHOICE OF INITIAL ANTIMICROBIAL DRUGS AND THE ROLE OF EXCHANGE TRANSFUSION[1]

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ABSTRACT Tollner U Pohlandt F Heinze F and Henrichs I (Department of Paediatrics Section of Neonatology University Hospital Ulm Federal Republic of Germany) Treatment of septicaemia in the newborn infant Choice of initial antimicrobial drugs and the role of exchange transfusion Acta Paediatr Scand 66 605 1977 —The therapeutic success of antibiotics used at the beginning of treatment and the effect of exchange transfusion in cases of septicaemia were tested in 22 newborn infants The clinical course of these patients was compared with the outcome of 11 newborn infants who received antibiotic treatment without exchange transfusion The following results were obtained 1) All 6 patients initially receiving antibiotics which were ineffective in vitro died In this group of patients the incidence of septic organ involvements (meningitis ventriculitis peritonitis) was significantly increased 2) Following exchange transfusion an impressive clinical improvement was consistently observed 3) In patients who had initially received effective antibiotics and exchange transfusion the lethality was significantly lower than in patients without exchange transfusion 4) Our bacteriological findings show that continuous monitoring of cultures from blood CSF and stool is necessary to choose the most effective antibiotic in the prevailing nosocomical circumstances

KEY WORDS Newborn infant septicaemia antibiotics exchange transfusion

Septicaemia of the newborn infant runs a rapid course and has a high lethality rate (9 15 23) It is accepted that its outcome is dependent on the efficacy of early antibiotic treatment although very few data are available to support this belief (5 17) Case reports dealing with successful use of exchange transfusion in septicaemia of the newborn infant (12 13) prompted us to apply this measure as ultima ratio in newborns with sepsis The unexpected improvement we observed led us to perform exchange transfusion earlier in the clinical course of rapidly deteriorating patients

[1] Presented in part at the 4th Symposium of Paediatric Intensive Care Medicine Mainz Oct 9–11 1975

The purpose of this paper is to demonstrate the effect of (*a*) initial choice of antibiotics and (*b*) exchange transfusion upon the lethality of neonatal septicaemia

PATIENTS AND METHODS

Patients

An exchange transfusion (e t) was performed as treatment for septicaemia [illegible]8 times in [illegible] newborns who were transferred to the intensive care unit from March 1974 to March 1975 The case histories of these patients were retrospectively analysed The data on gestational age birthweight and sex of the infants are shown in Fig 1 and Table 1 Prior to the onset of septicaemia 19 patients had developed respiratory distress syndrome Respiratory support was given by face mask CPAP in 16 infants and

been questioned by Shingwekar et al (24) and Meyer Dietrich (20) The Dubowitz scoring system has become very popular and its value has been proved in pre term infants (14 19) and in twins of dissimilar size (18) as well as in infants of African origin (3, 22) However misleadingly low scores might be found in infants born by extended breech delivery and in infants requiring prolonged resuscitation (14)

The increase in the precision in estimating gestational age by adding neurological tests to external characteristics is rather small if at all Therefore it is preferable to use only the external characteristics in routine work The external score is not influenced by the clinical state of the infants although small for gestational age infants are scored a little too low (5 11) The examination of external characteristics certainly upsets the sick infant much less than a neurological examination

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Submitted Dec 16 1976
Accepted Febr 16 1977

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Table 2 *Combinations of antimicrobial drugs which were used at the beginning of treatment for neonatal septicaemia during 1973–1975*

Combination of antibiotics No 1	
Gentamicin sulfate	5 mg/kg bodyweight during the first week of life given in 2 divided doses i m
	7 5 mg/kg bodyweight after the first week of life given in 3 divided doses i m
Oxacillin sodium	200 mg/kg bodyweight (max 300 mg/kg) given in 4 divided doses i v
Carbenicillin disodium	700 mg/kg bodyweight (max 1 000 mg/kg) given in 4 divided doses i v
Combination of antibiotics No 2	
Colistimethate sodium	16 mg/kg bodyweight given in 2 divided doses during the first week of life thereafter in 3 divided doses i m
Cephalothin sodium	60 mg/kg bodyweight given in 4 divided doses i v

after improvement during the first e t In 1? cases prednisolone was given 2 to 4 times at a dose of 5–10 mg per kg bodyweight to aid in control of septic shock (14 22 4 26 29) In 1973 3 patients received comparable doses of prednisolone

Statistical methods

The results were statistically validated by application of Fisher s exact test and the Mann Whitney rank sum test

RESULTS

Clinical outcome

From March 1974 to March 1975 22 newborns with severe septicaemia were treated of whom 11 survived (Fig 2)

Group 1 Five patients showed predominant cerebral lesions at autopsy which had caused death independently of septicaemia In one case an extensive haemorrhage into the falx cerebri subarachnoid space and both lateral ventricles was found In a second case wide spread pneumonia after aspiration of amniotic fluid and bilateral intraventricular haemorrhage were revealed Three further cases died of extensive cerebral haemorrhage

Table 3 *Organisms isolated from blood cultures of newborn infants Groups 2 and 3*

Figures within parentheses denote organisms resistant to initially used antibiotics

	1973 with out e t	1974/75 with e t
Escherichia coli	3	4
Enterobacter/klebsiella	3	11 (4)
Bacterium proteus	–	2 (1)
Pseudomonas aeruginosa	2	–
Enterococcus	–	1 (1)
β haemolytic Streptococcus Group A	–	1
Streptococcus viridans	–	1
Rods of coryneform group	1	–
Staphylococcus aureus	3	1

Group 2 Ten of 11 patients without localised involvements of septicaemia survived Gram negative bacteria were the causative organisms in 7 cases One patient died of sepsis due to enterococci resistant to the initially used antibiotics despite a second e t which was performed when he was moribund

Group 3 Five of 6 patients with localised involvements of sepsis died Four patients succumbed to meningitis and ventriculitis One patient who had received five e t on different days finally died and at autopsy was found to have intense purulent and fibrinous peritonitis forming a solid mass of the complete small intestine However the patient s condition improved during each e t and remained so for several hours afterwards

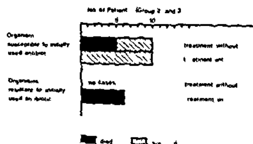


Fig 3 Result of treatment for neonatal septicaemia dependent on performance of exchange transfusion and susceptibility of isolated organisms (blood culture) to initially used antibiotics

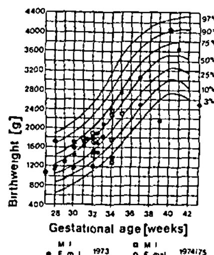


Fig 1 Newborn infants with septicaemia receiving different forms of treatment Distribution of birthweight gestational age and sex Local growth chart (21)

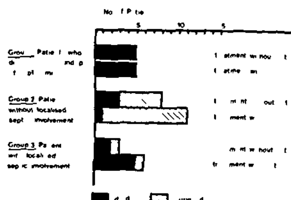


Fig 2 Effect of exchange transfusion on the outcome of neonatal septicaemia

by mechanical ventilation in 2 cases The patients were subdivided into three groups according to the following clinical symptoms and autopsy findings (Fig 2)

Group 1 Patients who died of nonseptic causes ($n=5$)

Group 2 Patients without localized septic involvement ($n=11$)

Group 3 Patients with localized septic involvement (meningitis ventriculitis peritonitis) ($n=6$)

The clinical course of patients who were treated with antibiotics and e t in 1974/75 was compared with the outcome of 16 newborns in 1973 who had received no e t (Fig 1 and Table 1) The patients with and without e t in both groups were comparable in regard to birthweight gestational age sex distribution history incidence of *respiratory distress syndrome course of illness change of* white cell count differential blood smear (27) antimicrobial treatment heparin and prednisolone dosage

Methods

The clinical diagnosis of septicaemia was made on the following grounds change of skin colour muscular hypotonia development of metabolic acidosis apneic spells and increased oxygen requirement Leukocytosis with a shift to the left and subsequent leukopenia supported the diagnosis A full discussion of the validity of these criteria has been reported previously (27)

Table 1 *Newborn infants with septicaemia*

	Median	Range
Birthweight (g)		
1973 (without e t)	1 740	1 150–3 650
1974/75 (with e t)	1 750	960–4 050
Gestational age (weeks)		
1973 (without e t)	32	28–43
1974/75 (with e t)	32	27–40
Sex distribution	Female	Male
1973 (without e t)	11	5
1974/75 (with e t)	12	10

After bloood samples cerebrospinal *fluid* stool and throat swabs had been obtained for bacteriological examination antibiotic therapy was started using arbitrarily either the combination of colistimethate sodium with cephalothin sodium or oxacillin sodium and carbenicillin disodium with gentamicin sulfate (Table 2)

To confirm the clinical diagnosis of septicaemia samples for culture were taken by venous and arterial puncture or from an umbilical venous catheter immediately after insertion (3) They were injected into brain heart infusion bottles (Mikrognost®)[1] and incubated at 37°C The susceptibility of the isolated bacteria was checked by a modified Kirby Bauer technique (4) using DST agar[2] against the following antibiotics (µg content of disc) oxacillin sodium (10) carbenicillin disodium (100) cephalothin sodium (30) colistimethate sodium (10) and gentamicin sulfate (10) As soon as the result was available—36 to 48 hours after initiation of treatment—the drug was changed if indicated All infants received about 100 U heparin/kg bodyweight per day which was added to the parenteral infusion Based on clinical considerations but not on coagulation studies heparin dosage was increased to 500 U in 6 arbitrarily chosen patients of the e t group and in 2 patients of the control group respectively in order to prevent consumption coagulopathy

E t was indicated whenever signs of septic shock developed A pale dirty skin colour accompanied by diminished microcirculation of skin and change from orange to greenish icterus served as predominant diagnostic criteria of septic shock aside from increasing oxygen requirement and metabolic acidosis (27) In 8 patients fresh heparinised blood in plastic bags was used containing 4 5 U heparin per ml The remaining 14 patients received ACD blood All infants received blood of their own blood group *The exchange transfusion was per*formed intermittently by 10 ml steps via umbilical vessel catheter The transfused volume amounted to about three times the blood volume of the patient but at most 450 ml Two patients received 2 and 5 e t respectively because signs of septic shock again developed some hours

[1] Biotest Serum Institut GmbH D-6000 Frankfurt/M
[2] Oxoid Deutschland GmbH D-4230 Wesel 1

Table 2 *Combinations of* [illegible] *which were used at the beginning of treatment for neonatal septicaemia during* [illegible]

Combination of antibiotics No 1	
Gentamicin sulfate	5 mg/kg bodyweight during the first week of life given in divided doses [illegible]
	7.5 mg/kg bodyweight after the first week of life given in 3 divided doses [illegible]
Oxacillin sodium	200 mg/kg bodyweight [illegible] 300 mg/kg given in [illegible] divided doses i.v.
Carbenicillin disodium	700 mg/kg bodyweight [illegible] 1000 mg/kg given in 4 divided doses i.v.
Combination of antibiotics No 2	
Colistimethate sodium	16 mg/kg bodyweight, given in divided doses during the first week of life, thereafter in 3 divided doses [illegible]
Cephalothin sodium	60 mg/kg bodyweight, given in 4 divided doses i.v.

after improvement during the first [illegible]. In 12 cases prednisolone was given 2 to 4 times at a dose of 5–10 mg per kg bodyweight to aid in control of septic shock ([illegible] 4 26 29). In 1973 3 patients received comparable [illegible] of prednisolone

Statistical methods

The results were statistically validated by application of Fisher's exact test and the Mann-Whitney rank sum test.

RESULTS

Clinical outcome

From March 1974 to March 1975 22 newborns with severe septicaemia were treated of whom 11 survived (Fig 2)

Group 1 Five patients showed predominant cerebral lesions at autopsy which had caused death independently of septicaemia. In one case an extensive haemorrhage into the falx cerebri, subarachnoid space and both lateral ventricles was found. In a second case widespread pneumonia after aspiration of amniotic fluid and bilateral intraventricular haemorrhage were revealed. Three further cases died of extensive cerebral haemorrhage

Table 3 [illegible]

Group 2 [illegible]

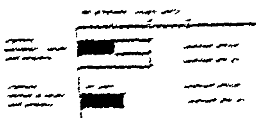


Fig 2 [illegible]

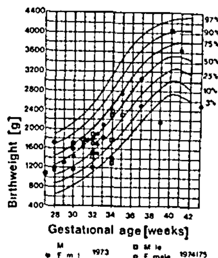


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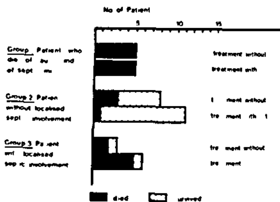


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ly improved humoral and cellular defense mechanisms We suppose that bacteria and endotoxins may be diluted and removed by e t Most recently reported animal experiments favour this hypothesis The survival rate of rabbits which had received endotoxin intravenously was significantly increased after an exchange transfusion (20) The removal of endotoxins might be followed by a diminished sensitivity of the peripheral vessels to catecholamines (14 29) Thus dilatation of these vessels improves the pale gray skin colour The spontaneous adjustment of metabolic acidosis and decreased oxygen requirement may also be a result of improved microcirculation It was striking that the clinical improvement which was well documented in some cases by arterial oxygen measurements began shortly after starting exchange transfusion However this response after 50–100 ml of exchange transfusion cannot be attributed exclusively to removal of endotoxins but might be produced by an endotoxin inhibitor in transfused blood Normal plasma is able to inhibit or detoxify endotoxin (16 25) A similar rapid increase of arterial oxygen pressure was most recently reported in premature infants with severe respiratory distress syndrome who had received exchange transfusion as a form of therapy (10) This response was thought to be a result of the removal of substances that might induce pulmonary hypoperfusion Heparin was not of significant value but could on the other hand not be shown to have increased the incidence of intracranial haemorrhage Prednisolone did not affect patient survival significantly Quite frequently neutropenia sometimes accompanied by the presence of immature granulocytes in the differential blood smear can be observed in neonatal septicaemia (27) Phagocytic capacity is attained at the myelocyte stage but is maximal at the polymorphonuclear stage (6) Transfusion of fresh blood might lead to an immediate increase of phagocytic capacity by both an enlargement of the granulocyte pool and by an increase of mature granulocytes

In addition there is evidence of humoral deficiency in the defence mechanisms of these infants Granulocytes of premature and term newborn infants have been shown to possess full phagocytic capacity when opsonization is intact (8 18) A deficiency of opsonization however has been proved against E coli Pseudomonas Staph aureus and Serratia marcescens in the serum of premature infants (8 18) A similar defect was shown against E coli and Serratia marcescens in the serum of term newborn infants (7) In addition the capacity of opsonization seems to correlate with the concentration of complement component C3 which again correlates with the age of gestation In newborn infants the amount of C3 and C5 in the serum is only about 50% of normal adult values (1)

IgM antibodies are needed for the activation of the complement system in the defence against Gram negative bacteria These antibodies are not available in newborn infants but the alternate pathway can be activated without antibodies It has been shown that in some newborn infants one component of this pathway is missing (2) Finally the serum of newborn infants seems to be devoid of a certain chemotactic factor (19) Transfusion of fresh blood might correct some or all of the factors causing the increased susceptibility of newborn infants to overwhelming infections

Experience concerning e t in the treatment of septicaemia in the newborn infant is not great Only short communications or case reports are available (12 13 28 30) This retrospective analysis may be less conclusive than a prospective controlled study but the data indicate that the therapeutic result in this life threatening illness is certainly improved by e t

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Choice of antibiotics at the beginning of treatment

In 1974/75 6 of 17 patients with septicaemia without intracerebral haemorrhage (Groups 2 and 3) were initially treated with a combination of antibiotics which proved to be ineffective in vitro (Fig 3 and Table 3) All 6 patients died The lethality rate in this group of patients who had received ineffective antibiotics during the first 36 to 48 hours of treatment was significantly increased compared with the effectively treated patients ($p<0\ 001$) Four of the 6 deceased newborn infants developed meningitis or ventriculitis Localised involvement of septicaemia occurred more frequently whenever initial treatment was not effective ($p=0\ 005$) All infants who had not received e t were treated by effective antibiotics at the beginning of therapy (Fig 3) However in three cases local manifestations of sepsis developed

Exchange transfusion

The clinical improvement during e t was consistently impressive Normal skin colour returned sometimes after only 50 ml of blood had been exchanged The frequency of apneic spells decreased and the oxygen content of incubator atmosphere or respirator air could be lowered and still keep the infants well oxygenated At times spontaneous breathing began during e t The frequently observed muscular hypotonia improved and spontaneous movements were resumed The metabolic acidosis which was always an accompanying finding could be corrected more easily In some cases in which serial measurements of arterial oxygen pressure and pH were made this improvement was well documented

Patients of groups 2 and 3 who had been treated with e t in 1974/75 were compared with those in 1973 who fell into the same groups but did not receive e t (Fig 3) All newborn infants in whom e t had been performed survived The case fatality rate was significantly reduced in patients who had received e t ($p=0\ 018$) This reduced lethality still holds after excluding those three patients without positive blood culture in whom the diagnosis of septicaemia was made on clinical grounds only ($p=0\ 040$)

DISCUSSION

Newborns especially premature infants who suffer from septicaemia only respond to early treatment because the disease runs such a rapid course (14 23) The necessarily blind antibiotic treatment used initially is therefore *of major importance in achieving therapeutic* success All our patients who had initially received ineffective antibiotics died

In 1974/75 6 of 21 isolated organisms were found to be resistant to the initial blind treatment There was no difference in the efficacy of any of the antibiotic combinations *colistimethate/cephalothin and oxacillin/car*benicillin/gentamicin in vitro In retrospect the combination of colistimethate/gentamicin would have been indicated for all bacteria isolated during this period

Treatment should be initiated as soon as the diagnosis is suspected and before the microorganism has been identified taking into account the bacteria likely to be encountered in the particular environment Information available in the literature concerning the spectra of antimicrobial drugs must be considered with caution since the reported bacterial spectra and sensitivity testing differ from one hospital to another perhaps because the use of different antibiotics in different treatment centers However continuous monitoring of organisms isolated from blood cerebrospinal fluid and stool including antibiotic susceptibility testing makes possible judicious selection of drugs likely to be effective in the initial blind treatment against the local bacterial spectrum

The mode of action of e t in the treatment of septicaemia remains hypothetical The following possibilities may be considered firstly rapid control of circulatory shock and second

ly improved humoral and cellular defense mechanisms We suppose that bacteria and endotoxins may be diluted and removed by e t Most recently reported animal experiments favour this hypothesis The survival rate of rabbits which had received endotoxin intravenously was significantly increased after an exchange transfusion (20) The removal of endotoxins might be followed by a diminished sensitivity of the peripheral vessels to catecholamines (14 29) Thus dilatation of these vessels improves the pale gray skin colour The spontaneous adjustment of metabolic acidosis and decreased oxygen requirement may also be a result of improved microcirculation It was striking that the clinical improvement which was well documented in some cases by arterial oxygen measurements began shortly after starting exchange transfusion However this response after 50–100 ml of exchange transfusion cannot be attributed exclusively to removal of endotoxins but might be produced by an endotoxin inhibitor in transfused blood Normal plasma is able to inhibit or detoxify endotoxin (16 25) A similar rapid increase of arterial oxygen pressure was most recently reported in premature infants with severe respiratory distress syndrome who had received exchange transfusion as a form of therapy (10) This response was thought to be a result of the removal of substances that might induce pulmonary hypoperfusion Heparin was not of significant value but could on the other hand not be shown to have increased the incidence of intracranial haemorrhage Prednisolone did not affect patient survival significantly Quite frequently neutropenia sometimes accompanied by the presence of immature granulocytes in the differential blood smear can be observed in neonatal septicaemia (27) Phagocytic capacity is attained at the myelocyte stage but is maximal at the polymorphonuclear stage (6) Transfusion of fresh blood might lead to an immediate increase of phagocytic capacity by both an enlargement of the granulocyte pool and by an increase of mature granulocytes

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Submitted May 29 1976
Accepted Febr 15 1977

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Acta Pædiatr Scand 66 611–616 1977

PAIN CRY IN FULL TERM ASPHYXIATED NEWBORN INFANTS CORRELATED WITH LATE FINDINGS

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ABSTRACT Michelsson K Sirvio P and Wasz Hockert O (II Department of Paediatrics and Department of Phonetics University of Helsinki Finland) Pain cry in full term asphyxiated newborn infants correlated with late findings Acta Paediatr Scand 66 611 1977.—115 pain induced cries from 45 full term newborn infants with pre and perinatal asphyxia were analyzed by sound spectrographic methods All the infants had signs of intrauterine asphyxia and Apgar score of 6 or less at 5 min The mean birth weight was 3 170 g The pain cries were recorded before the age of 8 days 83 % of the cries before 3 days of age The cry analysis was compared with the pain cries of 75 full term healthy newborn infants of corresponding birth weight and gestational age The results showed significant differences between the cries of the asphyxiated newborn and the healthy infants The duration of the phonation was shorter the maximum and minimum pitch of the fundamental frequency was significantly higher Bi phonation and vibrato occurred more often double harmonic break and glottal roll less often An increase in rising falling rising and flat types of melody was observed Retrospectively the cries were more abnormal if the infant was found to be neurologically damaged at the check up at 2–8 years

KEY WORDS Crying asphyxia newborn infant sound spectrography

Sound spectrographic methods have shown that the crying of healthy newborn infants has many specific features Different cry signals such as hunger and pain can be recognized both auditively and spectrographically (9 10 11) Since the beginning of the 1960s our study group has been analyzing the cry of both healthy neonates and those with different diseases and congenital abnormalities One part of our research programme was to study the cry characteristics of asphyxiated new born infants (4 5) with the purpose of discovering which characteristics of the pain cry correlate with the degree of asphyxia and the later findings for the child We have strong reasons to believe that cry analysis can be one more criterion in assessing not only the diagnosis but also the prognosis in the new born period

MATERIAL AND METHODS

The neonates The series comprised 45 newborn infants studied in 1966–71 at the Institute of Midwifery Hel sinki or in 1972–75 at the Children s Hospital Univer sity of Helsinki All the infants were full term with a gestational age of 38–42 weeks The mean birth weight was 3 170 g range 2 430–5 000 g

The infants were selected for the material according to two criteria signs of intrauterine asphyxia and the Apgar score at 5 min Changes in the foetal heart rate and dis colouring of the amniotic fluid were taken as signs of intrauterine asphyxia All the infants had an Apgar score at 5 min of 6 or less the score was 1–3 for 10 infants and 4–6 for 35 infants The score at one minute was 0–3 in 17 cases 4–6 in 27 cases and 7–10 in one case The Apgar scores at 15 min were 0–3 4–6 and 7–10 for 4 15 and 26 infants respectively

In the neonatal period many of the infants had neuro logical symptoms 6 had convulsions 3 cyanotic attacks 3 had signs of cerebral haemorrhage 6 of hypotonia 4 of rigidity and 2 of hyperirritability Two infants had tremor for several days Two of the infants had a slightly increased respiratory rate during the first day of life The blood sugar and the bilirubin were checked for all the

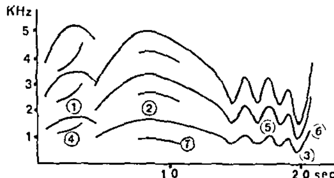


Fig 1 A schematic drawing of a sonagram displaying the following cry parameters 1 shift 2 maximum pitch 3 minimum pitch 4 bi phonation 5 vibrato 6 gliding and 7 double harmonic break

infants and showed no deviation from the normal limit at the time of the cry recordings

Controls The control series comprises 75 healthy full term newborn infants

Follow up At the check up examination at 2–8 years 11 of the children had marked neurological sequelae 5 had epileptic convulsions one of these was additionally severely mentally retarded Three more children had severe mental retardation and one of them had spastic hemiplegia as well Two children had moderate motor and mental retardation One child had developed hydrocephalus which was attributed to cerebral haemorrhage in the neonatal period Two of the infants had died in the newborn period with cerebral lesions according to the autopsy report

The mean birth weight of these two groups healthy and diseased was 3 150 g and 3 260 g respectively The Apgar scores were worse in those who had neurological sequelae later Of the 13 damaged infants 10 had Apgar scores below 6 at 1 5 and 15 min compared with 11 of the 32 children who were healthy at the time of the follow up

Recording of the cries The cries were tape recorded after a pinch on the upper arm or the ear thus called pain cry All the 115 signals were recorded before the age of 8 days Seventy were recorded during the first day of life all but 19 within 3 days of birth Forty five pain cries were analyzed from the 13 infants who had died or had neurological sequelae at check up and 70 from the 28 children who were healthy at the check up

Equipment and analyzing methods The pain induced cry signals were tape recorded on an Uher 4000 L tape recorder or a Sony TC 55 cassette tape recorder with an AKG 58 D 200 microphone

1–3 cries were analyzed from each infant usually the first phonation after the pinch The second and the third phonation of a few cries were also analyzed

The duration measurements were made with a mingograph (Elema Schonander Sweden) and the sound spectrographic analysis with a Sona Graph 6061 B (Kay Elemetrics Inc USA) The sound spectrograph provides a visualization of the cry signal a sonagram from which different cry parameters can be measured as shown schematically in Fig 1

A detailed explanation of the phonetic characteristics measured has been published by Wasz Hockert et al 1968 (9) and Michelsson 1971 (4) Therefore only a summary description is given here Fourteen cry attributes were measured The duration of the phonation the minimum and maximum pitch of the fundamental frequency the occurrence and maximum of shift We also noted the change in the melody type defined as falling rising falling rising falling-rising or flat In some of the cries no melody type was detectable A marked instability of the fundamental frequency was also noted as can be seen in Fig 2 We also observed the continuity and voicing of the signals and the glottal roll i e a low frequency voiced sound and the occurrence of the following parameters depicted on the schematic sonagram (Fig 1) bi phonation gliding vibrato and double harmonic break These characteristics were measured if the duration was at least 0 1 sec

RESULTS

The results of the analysis of the 115 cry signals of the 45 full term asphyxiated newborn infants are compared with the crying of 75 healthy full term newborn infants in Table 1 There were significant differences between the two series in the following cry characteristics

(*a*) The duration of the cry signals of the asphyxiated infants was significantly shorter

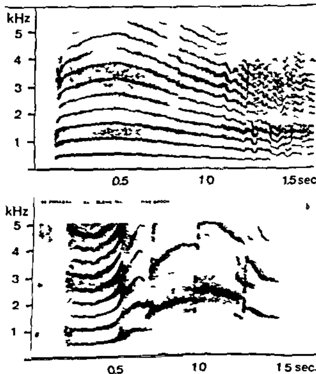


Fig 2 Pain cry of a healthy newborn infant (*a*) and of an infant with asphyxia at birth (*b*) The fundamental frequency of the cry in asphyxia is more high pitched and unstable

Table 1 *Results of the cry analysis of the 45 asphyxiated infants*
In separate columns the cry analysis with reference to later outcome

	Asphyxiated infants			
		According to later prognosis		
	All infants	Healthy	Damaged or dead	Controls
No of infants	45	32	13	75
No of cries analyzed	115	70	45	75
Duration sec Md	1 8[a]	2 3	1 2	2 8
Q_1–Q	1 1–3 5	1 5–4 5	0 7–2 0	1 8–3 4
Maximum pitch Hz Md	750[c]	640[c]	1 000[c]	610
Q_{25}–Q_{75}	510–1 510	490–1 350	550–1 750	570–690
Minimum pitch Hz Md	440	390	490	390
Q_{25}–Q_{75}	370–670	310–570	340–800	3 0–450
Occurrence of shift %	26	29	22	29
Max pitch of shift Hz Md	1 350	1 350	1 330	1 100
Melody type %				
Falling	23 }	24 }	27 }	63
Rising–falling	36 }	39 }	31 }	22
Rising	16 }	17 }	13 }	0
Falling rising	11 }	9 }	16 }	7
Flat	9 }	10 }	7 }	3
No type	5 }	1 }	11 }	5
Instable fund freq %	7[b]	6	9[b]	0
Continuous signals %	79	79	80	71
Voiced phonations %	68	63	76	63
Bi phonation %	34	30[c]	40[c]	1
Gliding %	5	4	7	0
Vibrato %	32	36	29	4
Double harmonic break %	20[c]	23	16	44
Glottal roll %	20	27[b]	9	51

$^{a}p<0.05$ $^{b}p<0.01$ $^{c}p<0.001$ Significance levels refer to differences between the characteristics in asphyxiated infants and controls

(*b*) The maximum and minimum pitch of the fundamental frequency was increased No healthy infant had a cry with a maximum pitch of over 1 000 Hz while 40 of the 115 cries of the asphyxiated infants exceeded this level

(*c*) The melody type which is mainly falling or rising–falling in the crying of healthy infants showed a significant increase in rising falling–rising and flat types of melody in the asphyxiated group Occasionally no melody type could be measured e g when the signal was very unstable or when a rising–falling–rising or falling–rising–falling type of melody occurred

(*d*) A significant increase was also noted in the occurrence of bi phonation vibrato double harmonic break and glottal roll

(*e*) A slight but not statistically significant increase was observed in the continuity and voicing of signals in the asphyxiated infants Gliding not seen in the crying of healthy neonates occurred in a few signals of the asphyxiated infants

(*f*) No significant change was seen in the occurrence of the shift part However in healthy neonates the shift part when present occurred in 4/5 at the beginning of the signal while in the asphyxiated infants it was at the beginning of the signal in about 1/3 of the cries

Table 1 also shows the results of the cry analysis of the asphyxiated infants in different columns according to the later findings Many of those characteristics which in the asphyxiated infants changed from the normal crying were still more marked when the asphyxiated infant died or was later found to be damaged for instance the falling and rising–falling type of melody decreased from 63% to 53% the

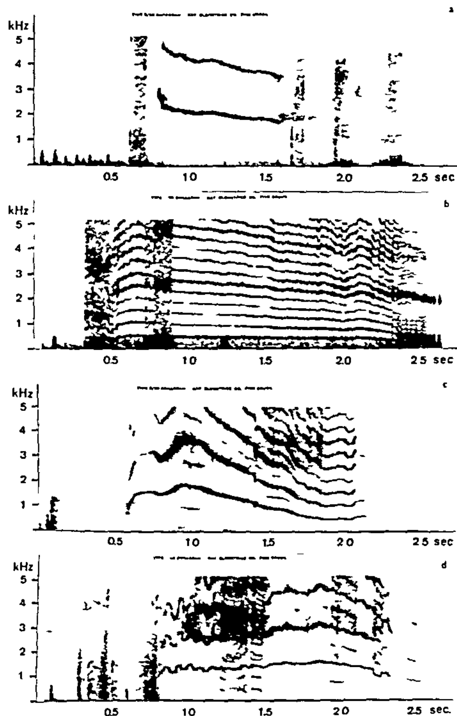


Fig 3 Pain cry signals recorded at the age of 1 day (*a* and *c*) and 8 days (*b* and *d*) The cries *a* and *b* are recorded from an infant healthy at follow up The cry was normal at 8 days The other infant developed CP and the cry at 8 days (*d*) was still very abnormal

occurrence of bi phonation increased from 30% to 40% the maximum pitch (Md) increased from 640 Hz to 1 000 Hz and the signals (Md) were much shorter 1 2 sec compared with 2 3 sec for healthy neonates

The table shows the significance levels matched to the normal series Comparison of the two groups of asphyxiated infants showed a statistically significant difference in the duration of the signals in the minimum and maximum pitch and in the occurrence of unstable signals and glottal roll

DISCUSSION

Sound spectrographic analysis of crying in infants has been studied systematically by our research group since the beginning of the 1960s when Wasz Hockert et al published their first analysis of different cry types such

as hunger pain and birth cries (11) Normative data for the crying of normal healthy infants were established in 1968 (9) Michelsson 1971 (4) showed that prematurely born infants cry differently from full term infants and that the differences were more marked the more premature the infant was For this reason we did not include premature babies in this study thus omitting differences in the cry characteristics due to prematurity

The criteria we selected for intrauterine asphyxia in this study were changes in the foetal heart rate and discolouring of the amniotic fluid We were aware of the problems of interpreting these symptoms but no better methods were available in 1966 when the first cries for this study were recorded The criterion for perinatal asphyxia was the Apgar score at 5 min since it has a better prognostic and diagnostic value than the one minute score (1)

The cry of infants with asphyxia has previously been studied by Michelsson (4) She showed that the crying of asphyxiated newborn infants had significant differences from that of healthy newborn infants These results concur with the findings of this work The purpose of this study was to ascertain whether the changes in some characteristics are more marked in newborn infants with severe asphyxia and whether more abnormal characteristics occur in those infants who were neurologically damaged at the follow up

Our study reveals that the crying of asphyxiated infants shows significant changes from the crying of healthy newborn infants The more high pitched cry found in infants with asphyxia is also audible to the ear In healthy neonates no cry exceeded 1000 Hz In the crying of the asphyxiated infants 35% of the cries exceeded this level If the child later was found to be damaged half of the cry signals had a pitch above 1000 Hz The minimum pitch was also significantly increased

In some of the phonations of the asphyxiated infants both the maximum and minimum pitch were well within the normal range but other abnormal characteristics occurred in these cries such as a change in the melody type or an occurrence of bi phonation or gliding We have attributed all these changes in the cry characteristics with a causal connection to the brain structure as they do not occur in the crying of infants with anomalies in the oral region such as cleft palate (6)

In the asphyxiated infants the shift part when present occurred more often at the middle or the end of the phonation a fact that has not attracted our attention hitherto The connection between the location of the shift part and the instability of the fundamental frequency is obvious and also the increase of vibrato can be connected with pitch instability The decrease in the occurrence of the low pitched glottal roll may be due to the overall shortening of the signals with a weaker and more abrupt termination of the cry

We observed in this study that the characteristics in the crying of asphyxiated infants which significantly differed from those of healthy infants were still more marked in those who were abnormal at check up Accordingly we feel that cry analysis can be one more expedient for diagnosing the possible brain damage resulting from severe asphyxia The measurements can be made more reliable by repeating the cry recordings This can be seen from Fig 3 which shows the cry characteristics of one infant who was normal at check up and those of another infant who had convulsions at the follow up In both cases the cries were recorded at 1 and 8 days of age It is noticeable how the cry becomes more normal as the child recovers but still has many abnormal qualities when the child is found to be damaged later

ACKNOWLEDGEMENT

This study was supported by the Sigrid Juselius Foundation

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Submitted Aug 12 1976
Accepted Febr 3 1977

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CELL MEDIATED IMMUNITY AND IMMUNOGLOBULIN LEVELS IN LIGHT FOR DATE INFANTS

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ABSTRACT Bhaskaram C Raghuramulu N and Reddy V (National Institute of Nutrition Hyderabad India) Cell mediated immunity and immunoglobulin levels in light for-date infants Acta Paediatr Scand 66 617 1977.—Cell mediated immune response and circulating levels of immunoglobulins were studied in 75 full term newborn babies The results showed that though the immunoglobulin levels were not altered cell mediated immunity was significantly depressed in infants with birth weight less than 2 500 g and this may result in lowered resistance to infection

KEY WORDS Cell mediated immunity immunoglobulins light for date infants

It is now well established that both humoral as well as cell mediated immune mechanisms are impaired in children with severe protein calorie malnutrition rendering them more susceptible to infections as shown by Reddy & Srikantia (7) and Bhaskaram & Reddy (2) Results of more recent studies conducted by Reddy et al (8) however have indicated that in mild and moderate degrees of protein calorie malnutrition as judged by growth retardation the same severe impairment in immunological responses are not seen This observation may have relevance not only to postnatal malnutrition but also to intra uterine malnutrition Ghosh (4) reported an increased frequency of infections in infants with low birth weight In India the incidence of low birth weight babies weighing less than 2 500 g is as high as 30% and a majority of them are full term light for-date infants There is little information regarding the functional status of such infants The present study was undertaken to assess the immunocompetence of light for date babies

SUBJECTS AND METHODS

Seventy five full term newborn babies were studied Gestational age was determined by the date of last menstrual period of the mother and confirmed by physical and neurological maturity of the newborn as suggested by Lubchenco (5) Cord blood samples were collected and the following investigations done

Table 1 *Cell mediated immunity in newborn babies*

Group	Birth weight (g)	No of babies	Total lymphocyte count/mm	Percentage of T cells	^{3}H thymidine incorporation: counts per min in test culture / counts per min in control culture
I	>2 500	6	3 ?44±740 9	56 8±1 43	?4 4
II	2 ?50–2 500	10	3 876±9?9 0	52 ?±3 26	10 9
III	< 2?50	13	3 144±648 0	36 7±3 01[b]	3 4[b]

Values are mean ±S E
[a] $p<0.05$
[b] $p<0.01$ compared with Group I

?0 77?875

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II	? 250–? 500	10	3 876±9?9 0	5? 3±3 ?6	10 9
III	<? 250	13	3 144±648 0	36 7±3 01*	3 4*

Values are mean ±S E
$p<0.05$
* $p<0.01$ compared with Group I

Table 2 *Immunoglobulin levels in newborn babies*

Group	Birth weight (g)	No of babies	Immunoglobulins mg/100 ml IgG	IgM	IgA
I	>2 500	45	1 478 0±117 1	12 5±1 28	0 6±0 20
II	2 250–2 500	17	1 751 0±149 1	10 9±2 19	0 5±0 10
III	<2 250	13	1 563 0±173 45	9 9±1 50	1 0±0 59

Values are mean ±S E

Total lymphocyte count was obtained by making total and differential leukocyte counts The cell mediated immune response was assessed by T lymphocyte count as measured by rosette formation technique described by Wybran et al (9) and ^{3}H thymidine incorporation into lymphocyte cultures following PHA stimulation described in the method reported by Bhaskaram & Reddy (2) The results are expressed as the ratio of counts per minute in test culture and control culture

Serum IgG IgM and IgA were determined by radial immunodiffusion technique of Mancini (6)

RESULTS

The infants were divided into three groups based on the birth weight There were 45 infants weighing more than 2 500 g and 17 infants weighing between 2 250 and 2 500 g Thirteen infants weighed less than 2 250 g and only 3 of them were below 2 000 g

The mean level of the ratio of ^{3}H thymidine incorporation in the test to the control culture and percentage of T lymphocytes were 24 4 and 56 8 respectively in babies weighing more than 2 500 g Values for ^{3}H thymidine incorporation into lymphocytes of babies weighing less than 2 500 g were significantly lower The number of T cells however was significantly lower only in those weighing less than 2 250 g as compared with infants with birth weights above 2 500 g (as shown in Table 1) The total lymphocyte counts did not differ in the three groups of newborns

The concentration of IgG in cord blood was much higher than that of IgM and IgA Levels of all three immunoglobulins in infants with birth weights less than 2 500 g as shown in Table 2 were similar to those in infants weighing more than 2 500 g

DISCUSSION

The results of the present study indicate that cell mediated immunity is significantly depressed in light for date infants—an observation similar to that reported by Chandra (3) The immunoglobulin levels on the other hand did not seem to be related to the birth weight of the infants This observation however is different from that of Chandra who found a significant decrease in IgG levels of infants with low birth weights Infants studied by Chandra however constitute a heterogeneous group and included premature babies It has been demonstrated by Berg (1) that IgG concentration is well correlated to gestational age but not to birth weight of infants The results presented here indicate that the immunoglobulin levels are not altered in full term light for date infants It must be pointed out however that these infants did not show severe intrauterine growth retardation There were only 3 infants weighing less than 2 000 g

These results suggest that all immunological functions are not equally sensitive to fetal malnutrition and parallel observations in older children with malnutrition as reported by Reddy et al (8) They do however show that infants with birth weights less than 2 500 g are likely to be at increased risk since they suffer from at least one immunological defect which may result in lowered resistance to infection

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Submitted Aug 3 1976
Accepted Febr 1 1977

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Acta Pædiatr Scand 66 621–624 1977

THE EFFECT OF CHOLESTYRAMINE ON SERUM LIPIDS AND PLATELET AGGREGATION OF HYPERCHOLESTEROLEMIC CHILDREN (TYPE II A) WHILE ON HIGH LINOLEIC ACID DIET

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ABSTRACT Fernandes J Dijkhuis-Stoffelsma R and Grose W F A (Department of Paediatrics Sophia Children s Hospital and Neonatal Unit Academic Hospital Erasmus University Rotterdam the Netherlands) The effect of cholestyramine on serum lipids and platelet aggregation of hypercholesterolemic children (type II A) while on high linoleic acid diet Acta Paediatr Scand 66 621 1977.—25 children with familial hypercholesterolemia (type II A) were treated with cholestyramine or placebo in a cross over study during 2 periods of each 10 weeks The medication was added to a high linoleic acid diet which had been started at least 1 year earlier Serum lipids and platelet aggregation were investigated at the end of the 2 periods On cholestyramine serum cholesterol levels decreased significantly whereas the linoleate and oleate content of cholesterylesters and serum triglycerides did not change systematically Platelet aggregation time measured with a filtragometer did not systematically change either

KEY WORDS Hypercholesterolemia cholestyramine high linoleic acid diet serum cholesterol serum triglycerides cholesterylesters platelet aggregation

Familial hypercholesterolemia Type II is associated with an increased risk of premature atherosclerosis (12 15) Reduction of the high serum cholesterol levels by a high linoleic acid diet (3) has successfully delayed death from occlusive vascular disease (13) and the results were more favourable the longer the dietary treatment had been applied (2) Early diagnosis and treatment in the pediatric age has therefore been recommended (5) Another issue has been the effect of a reduction of serum cholesterol by medication without diet (16) or by medication in addition to diet (5) on the life expectancy of children with familial hypercholesterolemia To approach this problem the mechanism of the atherosclerotic process has been more closely investigated Platelet adhesiveness might be an important factor in initiating atheromatous plaque formation and it has been demonstrated that linoleic acid enriched diets significantly reduce platelet aggregatability (4 7) Furthermore there is some evidence that a high linoleic acid concentration of serum cholesterylesters might decrease the tendency to human atherosclerosis (11) whereas a high oleic acid concentration could have the opposite effect (6) We focussed our attention on these aspects of the hypercholesterolemic state in those children in whom the dietary induced decrease of serum cholesterol was insufficient We administered cholestyramine or placebo in addition to the diet in order to know whether a further decrease of serum cholesterol by cholestyramine would be accompanied by changes in platelet aggregation and fatty acid composition of cholesterylesters

Table 1 *Serum cholesterol during high linoleic acid diet with cholestyramine or placebo added*

Serumcholesterol mmol/l (mean ±S E M) Q=Questran P=placebo 1 and 2=periods of 10 weeks

	1	2
$n=11$	P 7 3±0 28	Q 6 1±0 31
$n=14$	Q 5 8±0 44	P 7 1±0 45

MATERIAL AND METHODS

The criteria for diagnosing hypercholesterolemia Type II were as follows all the children had serum cholesterol concentrations ranging from 7–11 mM Serum triglyceride concentrations were normal (<1 50 mM) Obesity and hypertension were not present Renal disease hypothyroidism diabetes mellitus and metabolic diseases were excluded

One parent of each of the children had hypercholesterolemia or had already died from cardiovascular disease We selected 25 children who had kept to a high linoleic acid diet during 1 to 3 years and whose serum cholesterol had decreased to a steady level The ages of the children were 4 to 14 years Two groups of 11 and 14 children were formed comparable as regards the patients age the severity of hypercholesterolemia and the linoleic acid content of the diet Siblings were present in both groups Cholestyramine was administered to one group placebo to the other After 10 weeks the medication of the 2 groups was reversed for another 10 weeks period The patients were not aware of the placebo prescription They consumed cholestyramine or placebo in a dose of 0 3 to 0 6 g per kg body weight per day This dose was divided in two parts for ingestion at breakfast and dinner Cholestyramine (Questran) and placebo were supplied on our request by Mead Johnson USA They contained 5 mg riboflavin per 4 g sachet This vitamin was added for its easy detection in the urine The qualitative assay of riboflavin in the urine (present or absent) thus facilitated the investigation of drug adherance of the patients in cases of doubt The patients diets during the 2 experimental periods remained the same as before The total fat content was approximately 30 cal% the linoleic acid content was 8 to 20 cal% the cholesterol intake was not more than 195 mg per day Medical examination of the patients with measurement of length and weight inquiry into adherance to the diet and the medication and laboratory investigations were carried out at the end of each 10 weeks period For each control visit the children were instructed to take a standard low fat breakfast in order to prevent prolonged fasting This low fat breakfast would presumably not influence the serum cholesterol level and platelet aggregation

METHODS

Serum cholesterol was determined according to Huang et al (9)

Serum triglycerides were determined according to Soloni (14)

For the fatty acid composition of cholesterylesters the procedure of Beukers et al (1) was followed using cholesteryl pentadecanoate as an internal standard in the organic solvent The cholesterylester band made visible with iodine vapor was scraped off and extracted with a chloroform methanol 1 1 mixture After evaporation of the solvents the esters were saponified with methanolic KOH at 65 C Cholesterol was removed by pentane extraction the water fraction was acidified and the free fatty acids were isolated by pentane extraction The solvent was evaporated and the acids were esterified with diazomethane in ether

The band on the thin layer plate containing the triglycerides was also isolated and extracted with a chloroform methanol 1 1 mixture containing pentadecanoic acid as a standard This extract was submitted to a similar procedure as described above

The gaschromatographic analyses were performed on a Hewlett Packard 402 gaschromatograph using dual 2 m 4 mm ID glass columns packed with 10% EGA on chromosorb W starting temperature 150°C final temperature 210°C program rate 2 C per min flash heater 170°C flame ionisation detector temperature 240°C Calculations were done with an on line Hewlett Packard 3352B computer

Platelet aggregation time was measured by the filtragometer procedure of Hornstra et al (7 8) With this method the aggregation of platelets in flowing blood is assessed by drawing blood via a polyethylene cannula from an anticubital vein through a microfilter into a motor driven syringe (diameter of the filter 2 3 mm pore size 20×70 µm flowrate of the blood 2 ml per min)

The filter s pores permit passage of red and white bloodcells and platelets but platelet aggregates occlude the pores Obstruction of the filter by platelet aggregates is reflected by an increasing difference between the blood pressures at both sides of the filter Aggregation time is the interval in seconds between venipuncture and obstruction of the filter (pressure difference at both sides of the filter ≥5 mmHg)

Riboflavin in the freshly voided morning urine was fluorometrically estimated at 524 nm in a Vitatron UFD 100 photometer with excitation at 443 nm A urine fluorescence of 30% or more in proportion to a standard solution of 10 mg/l riboflavin was considered to be positive

RESULTS

From interviews with the parents and from incidental testing of the patients urine for the presence of riboflavin it was deduced that the patients adherance to diet and medication was good Side effects of cholestyramine were similar to those reported by others Nausea or sensation of fullness could be mitigated by allowing 2 or 3 days for gradually increasing cholestyramine to the therapeutic dose of 0 3

to 0 6 g/kg/day Side effects such as steator rhea and low serum folate levels (16) were not investigated as the Questran doses were low and the medication lasted only 10 weeks

The serum cholesterol data are summarized in Table 1 The mean cholesterol concentra tion after 10 weeks of placebo added to the diet was 7 2 mmol/l after 10 weeks of Ques tran 6 0 mmol/l The difference between these data is highly significant $p<0\ 001$

The fatty acid composition of cholesteryles ters did not systematically change during the experiment for this reason only the means of the most important fatty acids i e linoleic acid and oleic acid and the oleic acid/linoleic acid ratio are summarized in Table 2

The serum triglyceride levels were normal in all children and did not systematically change during the experiment The linoleic acid and oleic acid percentages of serumtri glycerides and the oleic acid/linoleic acid ratio did not systematically change either (data not given) The data of platelet aggregation times are summarized in Table 3 The aggregation times were transformed to their logarithms for statistical treatment No systematic dif ferences were found between the two periods Other parameters for aggregation and disag gregation which can also be obtained from the filtragometry method of Hornstra (8) are not given as the number of patients in whom these data could be measured was too small

Table 2 *Composition of serum cholesteryles ters during high linoleic acid diet with choles tyramine or placebo added*

Fatty acid percentage of serum cholesterylesters (mean ±S E M) C18 1=oleic acid C18 ? linoleic acid Ratio =C18 1/C18 1 and =periods of 10 weeks

		1	?
		P	Q
n=11	C18 1	0±1 9	17±0 6
	C18 ?	48±? 4	50±3 1
	Ratio	0 44±0 07?	0 36±0 0 8
		Q	P
n=14	C18 1	15±0 5	14±0 6
	C18	54±1 4	58±1 5
	Ratio	0 8±0 015	0 5±0 015

Table 3 *Platelet aggregation time during high linoleic acid diet with cholestyramine or place bo added*

Log platelet aggregation time (mean ±S E M) Q=Ques tran P=placebo 1 and 2=periods of 10 weeks

	1	2
n=11	P ? 1?±0 114	Q 2 38±0 065
n=13	Q ? 09±0 084	P ? 10±0 10?

DISCUSSION

Addition of cholestyramine to a high linoleic acid diet successfully decreased the serum cholesterol concentrations of children with familial hypercholesterolemia (5) (Table 1) It is not known whether this has a favourable influence on the increased tendency to athero sclerotic disease of these patients We there fore investigated some factors which are in volved in atheromatous plaque formation One of these is platelet aggregatability which is known to be decreased by linoleic acid en riched diets (4 7) We did not find that the addition of cholestyramine to the diet had any effect on platelet aggregation time (Table 3)

It must be stressed however that our re sults were obtained with a filtragometer with a filter which has approximately the same pore size as the apparatus used in the original pub lications (7) and by others (4) but a smaller diameter Our experiments therefore need am plification using a larger group of patients and filtragometers with different filters Another important parameter for the atherosclerotic tendency seems to be the composition of cho lesterylesters Kingsbury et al (11) showed that patients with a reduced concentration of linoleic acid (<35%) subsequently had a higher incidence of myocardial infarction Gottenbos (6) suggested that not only the favourable effect of cholesterylester linoleate but also the unfavourable effect of cholesteryl ester oleate determine the tendency for athero sclerosis

In our study cholestyramine did not sys tematically influence cholesterylester linoleate

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$n=14$	Q 5 8±0 44	P 7 1±0 45

MATERIAL AND METHODS

The criteria for diagnosing hypercholesterolemia Type II were as follows all the children had serum cholesterol concentrations ranging from 7–11 mM Serum triglyceride concentrations were normal (<1 50 mM) Obesity and hypertension were not present Renal disease hypothyroidism diabetes mellitus and metabolic diseases were excluded

One parent of each of the children had hypercholesterolemia or had already died from cardiovascular disease We selected 25 children who had kept to a high linoleic acid diet during 1 to 3 years and whose serum cholesterol had decreased to a steady level The ages of the children were 4 to 14 years Two groups of 11 and 14 children were formed comparable as regards the patients age the severity of hypercholesterolemia and the linoleic acid content of the diet Siblings were present in both groups Cholestyramine was administered to one group placebo to the other After 10 weeks the medication of the 2 groups was reversed for another 10 weeks period The patients were not aware of the placebo prescription They consumed cholestyramine or placebo in a dose of 0 3 to 0 6 g per kg body weight per day This dose was divided in two parts for ingestion at breakfast and dinner Cholestyramine (Questran) and placebo were supplied on our request by Mead Johnson USA They contained 5 mg riboflavin per 4 g sachet This vitamin was added for its easy detection in the urine The qualitative assay of riboflavin in the urine (present or absent) thus facilitated the investigation of drug adherance of the patients in cases of doubt The patients diets during the 2 experimental periods remained the same as before The total fat content was approximately 30 cal% the linoleic acid content was 8 to 20 cal% the cholesterol intake was not more than 195 mg per day Medical examination of the patients with measurement of length and weight inquiry into adherance to the diet and the medication and laboratory investigations were carried out at the end of each 10 weeks period For each control visit the children were instructed to take a standard low fat breakfast in order to prevent prolonged fasting This low fat breakfast would presumably not influence the serum cholesterol level and platelet aggregation

METHODS

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Serum triglycerides were determined according to Soloni (14)

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RESULTS

From interviews with the parents and from incidental testing of the patients urine for the presence of riboflavin it was deduced that the patients adherance to diet and medication was good Side effects of cholestyramine were similar to those reported by others Nausea or sensation of fullness could be mitigated by allowing 2 or 3 days for gradually increasing cholestyramine to the therapeutic dose of 0 3

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Table 2 *Composition of serum cholesterylesters during high linoleic acid diet with cholestyramine or placebo added*

Fatty acid percentage of serum cholesterylesters (mean ±S E M) C18 1=oleic acid C18 2 linoleic acid Ratio =C18 1/C18 2 1 and 2=periods of 10 weeks

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DISCUSSION

Addition of cholestyramine to a high linoleic acid diet successfully decreased the serum cholesterol concentrations of children with familial hypercholesterolemia (5) (Table 1) It is not known whether this has a favourable influence on the increased tendency to atherosclerotic disease of these patients We therefore investigated some factors which are involved in atheromatous plaque formation One of these is platelet aggregatability which is known to be decreased by linoleic acid enriched diets (4 7) We did not find that the addition of cholestyramine to the diet had any effect on platelet aggregation time (Table 3)

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Log platelet aggregation time (mean ±S E M) Q=Questran P=placebo 1 and 2=periods of 10 weeks

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DISCUSSION

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Table 1 *Serum cholesterol during high linoleic acid diet with cholestyramine or placebo added*

Serumcholesterol mmol/l (mean ±S E M) Q=Questran P=placebo 1 and 2=periods of 10 weeks

	1	2
n=11	P 7 3±0 28	Q 6 1±0 31
n=14	Q 5 8±0 44	P 7 1±0 45

MATERIAL AND METHODS

The criteria for diagnosing hypercholesterolemia Type II were as follows all the children had serum cholesterol concentrations ranging from 7–11 mM Serum triglyceride concentrations were normal (<1 50 mM) Obesity and hypertension were not present Renal disease hypothyroidism diabetes mellitus and metabolic diseases were excluded

One parent of each of the children had hypercholesterolemia or had already died from cardiovascular disease We selected 25 children who had kept to a high linoleic acid diet during 1 to 3 years and whose serum cholesterol had decreased to a steady level The ages of the children were 4 to 14 years Two groups of 11 and 14 children were formed comparable as regards the patients age the severity of hypercholesterolemia and the linoleic acid content of the diet Siblings were present in both groups Cholestyramine was administered to one group placebo to the other After 10 weeks the medication of the 2 groups was reversed for another 10 weeks period The patients were not aware of the placebo prescription They consumed cholestyramine or placebo in a dose of 0 3 to 0 6 g per kg body weight per day This dose was divided in two parts for ingestion at breakfast and dinner Cholestyramine (Questran) and placebo were supplied on our request by Mead Johnson USA They contained 5 mg riboflavin per 4 g sachet This vitamin was added for its easy detection in the urine The qualitative assay of riboflavin in the urine (present or absent) thus facilitated the investigation of drug adherance of the patients in cases of doubt The patients diets during the 2 experimental periods remained the same as before The total fat content was approximately 30 cal% the linoleic acid content was 8 to 20 cal% the cholesterol intake was not more than 195 mg per day Medical examination of the patients with measurement of length and weight inquiry into adherance to the diet and the medication and laboratory investigations were carried out at the end of each 10 weeks period For each control visit the children were instructed to take a standard low fat breakfast in order to prevent prolonged fasting This low fat breakfast would presumably not influence the serum cholesterol level and platelet aggregation

METHODS

Serum cholesterol was determined according to Huang et al (9)

Serum triglycerides were determined according to Soloni (14)

For the fatty acid composition of cholesterylesters the procedure of Beukers et al (1) was followed using cholesteryl pentadecanoate as an internal standard in the organic solvent The cholesterylester band made visible with iodine vapor was scraped off and extracted with a chloroform methanol 1 1 mixture After evaporation of the solvents the esters were saponified with methanolic KOH at 65 C Cholesterol was removed by pentane extraction the water fraction was acidified and the free fatty acids were isolated by pentane extraction The solvent was evaporated and the acids were esterified with diazomethane in ether

The band on the thin layer plate containing the triglycerides was also isolated and extracted with a chloroform methanol 1 1 mixture containing pentadecanoic acid as a standard This extract was submitted to a similar procedure as described above

The gaschromatographic analyses were performed on a Hewlett Packard 402 gaschromatograph using dual ? m 4 mm ID glass columns packed with 10% EGA on chromosorb W starting temperature 150°C final temperature 210°C program rate 2 C per min flash heater 1/0°C flame ionisation detector temperature 240°C Calculations were done with an on line Hewlett Packard 3357B computer

Platelet aggregation time was measured by the filtragometer procedure of Hornstra et al (7 8) With this method the aggregation of platelets in flowing blood is assessed by drawing blood via a polyethylene cannula from an anticubital vein through a microfilter into a motor driven syringe (diameter of the filter 2 3 mm pore size ?0× 0 μm flowrate of the blood 2 ml per min)

The filter s pores permit passage of red and white bloodcells and platelets but platelet aggregates occlude the pores Obstruction of the filter by platelet aggregates is reflected by an increasing difference between the blood pressures at both sides of the filter Aggregation time is the interval in seconds between venipuncture and obstruction of the filter (pressure difference at both sides of the filter ≥5 mmHg)

Riboflavin in the freshly voided morning urine was fluorometrically estimated at 524 nm in a Vitatron UFD 100 photometer with excitation at 443 nm A urine fluorescence of 30% or more in proportion to a standard solution of 10 mg/l riboflavin was considered to be positive

RESULTS

From interviews with the parents and from incidental testing of the patients urine for the presence of riboflavin it was deduced that the patients adherance to diet and medication was good Side effects of cholestyramine were similar to those reported by others Nausea or sensation of fullness could be mitigated by allowing 2 or 3 days for gradually increasing cholestyramine to the therapeutic dose of 0

to 0 6 g/kg/day Side effects such as steatorrhea and low serum folate levels (16) were not investigated as the Questran doses were low and the medication lasted only 10 weeks

The serum cholesterol data are summarized in Table 1 The mean cholesterol concentration after 10 weeks of placebo added to the diet was 7 2 mmol/l after 10 weeks of Questran 6 0 mmol/l The difference between these data is highly significant $p<0\ 001$

The fatty acid composition of cholesterylesters did not systematically change during the experiment for this reason only the means of the most important fatty acids i e linoleic acid and oleic acid and the oleic acid/linoleic acid ratio are summarized in Table 2

The serum triglyceride levels were normal in all children and did not systematically change during the experiment The linoleic acid and oleic acid percentages of serumtriglycerides and the oleic acid/linoleic acid ratio did not systematically change either (data not given) The data of platelet aggregation times are summarized in Table 3 The aggregation times were transformed to their logarithms for statistical treatment No systematic differences were found between the two periods Other parameters for aggregation and disaggregation which can also be obtained from the filtragometry method of Hornstra (8) are not given as the number of patients in whom these data could be measured was too small

Table 2 *Composition of serum cholesterylesters during high linoleic acid diet with cholestyramine or placebo added*

Fatty acid percentage of serum cholesterylesters (mean ±S E M) C18 1=oleic acid C18 2 linoleic acid Ratio =C18 1/C18 *1* and 2=periods of 10 weeks

		1	2
		P	Q
n=11	C18 1	20±1 9	17±0 6
	C18	48±2 4	50±3 1
	Ratio	0 44±0 072	0 36±0 078
		Q	P
n=14	C18 1	15±0 5	14±0 6
	C18	54±1 4	58±1 5
	Ratio	0 28±0 015	0 25±0 015

Table 3 *Platelet aggregation time during high linoleic acid diet with cholestyramine or placebo added*

Log platelet aggregation time (mean ±S E M) Q=Questran *P*=placebo *1* and 2=periods of *10 weeks*

	1	2
n=11	P 2 12±0 114	Q 2 38±0 065
n=13	Q 2 09±0 084	P 2 10±0 103

DISCUSSION

Addition of cholestyramine to a high linoleic acid diet successfully decreased the serum cholesterol concentrations of children with familial hypercholesterolemia (5) (Table 1) It is not known whether this has a favourable influence on the increased tendency to atherosclerotic disease of these patients We therefore investigated some factors which are involved in atheromatous plaque formation One of these is platelet aggregatability which is known to be decreased by linoleic acid enriched diets (4 7) *We did not find that the* addition of cholestyramine to the diet had any effect on platelet aggregation time (Table 3)

It must be stressed however that our results were obtained with a filtragometer with a filter which has approximately the same pore size as the apparatus used in the original publications (7) and by others (4) but a smaller diameter Our experiments therefore need amplification using a larger group of patients and filtragometers with different filters Another important parameter for the atherosclerotic tendency seems to be the composition of cholesterylesters Kingsbury et al (11) showed that patients with a reduced *concentration* of linoleic acid (<35%) subsequently had a higher incidence of myocardial infarction Gottenbos (6) *suggested* that not only the favourable effect of cholesterylester linoleate but also the unfavourable effect of cholesteryl ester oleate determine the tendency for atherosclerosis

In our study cholestyramine did not systematically influence cholesterylester linoleate

and oleate concentrations during a high linoleic acid diet (Table 2) We have no explanation for the fact that the 2 groups matched for age and serum cholesterol levels of the patients and the linoleic acid content of the diets had different mean percentages of linoleic acid of cholesterylesters (48 and 54% respectively) These percentages increased in both groups (to 50 and 58% respectively) despite the cross over design of the study This increase of the linoleic acid percentages in both groups is most likely due to a time effect

It has previously been found that another cholesterol lowering agent (Clofibrate) reduced linoleate and increased oleate concentrations of cholesterylesters (10) It might be argued that cholestyramine though not influencing the oleate/linoleate proportion of cholesterylesters could augment the effect of a high linoleic acid diet by decreasing the total amount of cholesterol and thus the amount of cholesterylester oleate

From our data and those of others we may conclude the following cholestyramine, added to a high linoleic acid diet augments the cholesterol lowering effect of the diet but has no effect on platelet aggregation nor on the relative linoleate and oleate content of cholesterylesters and triglycerides It use could be considered in those children who remain severely hypercholesterolemic despite the ingestion of a high linoleic acid diet

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Acta Pædiatr Scand 66 625–634 1977

REVIEW ARTICLE

PREVENTION OF KERNICTERUS BASED ON RECENT PROGRESS IN BILIRUBIN CHEMISTRY

R BRODERSEN

From the Institute of Medical Biochemistry University of Aarhus Aarhus Denmark

ABSTRACT Brodersen R (Institute of Medical Biochemistry University of Aarhus, Aarhus Denmark) Prevention of kernicterus based on recent progress in bilirubin chemistry Acta Paediatr Scand 66 625 1977 —A review is presented of recent progress in bilirubin chemistry its binding to albumin displacement by drugs and the mechanism of phototherapy Quantitative formulations of the effect of albumin dosage of varying pH and of fatty acids result in a diagram which may be tried as an aid to indications for therapy

KEY WORDS Albumin alkali therapy bilirubin displacement bilirubin isomers exchange transfusion fatty acids kernicterus phototherapy

Free bilirubin from plasma may penetrate the basal ganglia and cause yellow staining kernicterus or may in non fatal cases give lasting brain damage of varying severity Avoidance of neurotoxic levels of free bilirubin is accordingly an important pediatric concern and may be aided by recently acquired knowledge of the chemistry of bilirubin and its binding to albumin as outlined in the present review

Chemical structure of bilirubin Solubility and neurotoxicity

X ray crystallographic studies by Bonnett et al (6) have shown that crystalline bilirubin the acid form has the structure shown in Fig 1 The side chains are in the IX-α position and the double bonds adjacent to the outer rings are in the Z-configuration (compare Fig 4 which shows the E configuration) Intramolecular hydrogen bonds (stippled lines in Fig 1) saturate the hydrophilic groups of the molecule leaving no affinities for attachment of water This substance the acid form of bilirubin IX-α(Z) is therefore nearly insoluble in water

In an alkaline medium the hydrogen bonds are opened forming the divalent anion (Fig 2) which has several hydrophilic groups i e two carboxylate four pyrrol N and two C=O and forms soluble salts At pH 7 4 the solubility of bilirubin IX α(Z) is low of the order of 0 1 μmol/l (18) increasing steeply in more alkaline media

According to McDonagh & Assisi (32) the bilirubin anion in aqueous solution is cleaved slowly at the central methylene bridge and is reunited at random forming a mixture containing two additional isomers III α and XIII α (abbreviated formulae shown in Fig 3*b* c) These are found together with bilirubin IX α(Z) in some commercial bilirubin preparations (31) while only one of these the IX α(Z) isomer has been found *in vivo*

Recent work in Heirwegh s laboratory (3 4 5 24) has shown that small amounts of three

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Accepted March 28 1977

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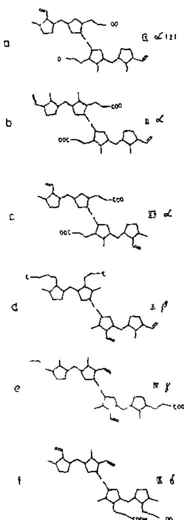


Fig 3 Abbreviated formulae omitting hydrogen bonds of bilirubin isomers Bilirubins III-α and XIII-α are found in some commercial preparations but not in vivo The IX β IX γ and IX-δ isomers are present in small amounts in the human body are soluble excreted without conjugation and are presumably non toxic

around 400 μmol/l b_0 is of the order of 50 nmol/l Concentrations of free bilirubin IX α(Z) higher than this figure could cause kernicterus

A somewhat better definition of the safe upper limit of free bilirubin concentration may be obtained from the work of Wennberg et al (45) These investigators used the peroxidase technique described by Jacobsen & Wennberg (30) on a large number of serum samples at 27° and found that kernicterus did not occur at a free bilirubin concentration less than 20 nmol/l Correction to 37 utilizing the temperature dependence of the binding constant as determined by Jacobsen (28) results in multiplication of this figure by 2 5 giving 50 nmol/l as above These findings suggest that the safe upper limit of free bilirubin IX α(Z) concentration is indeed about 50 nmol/l

It is interesting to note that this level of free bilirubin is reached when 78% of the albumin high affinity site is saturated with bilirubin Full saturation of the high affinity site would give a very high value of free bilirubin and is not thermodynamically possible When binding to secondary sites is taken into account it is found (14 15) that one mole of albumin is capable of carrying a maximum of 1 0 mole of bilirubin IX α(Z) If an occasional bilirubin concentration higher than the molar concentration of albumin is encountered in the clinic the presence of soluble bilirubin isomers or conjugates should be considered

Analytical methods for determination of the concentration of free bilirubin IX α(Z) in undiluted blood plasma or serum are not available at present (10) The Sephadex technique is not specific since bilirubin conjugates are adsorbed the soluble bilirubin isomers are probably also adsorbed The peroxidase method (30) is less sensitive to errors caused by the presence of other bilirubins A small

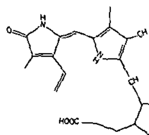


Fig 4 Bilirubin IX-α(E) (one half of the molecule shown) formed by irradiation with blue light of bilirubin IX α(Z) bound to serum albumin through rotation of the outer ring at the double bond This isomer is also soluble and presumably non toxic

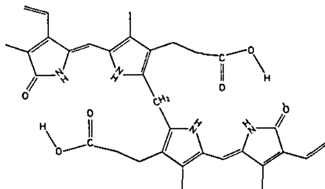


Fig 1 The main bile pigment in the human body bilirubin IX α(Z) in its acid form An array of intramolecular hydrogen bonds (stippled lines) is characteristic for this substance and is essential for its insolubility and its neurotoxicity

other bilirubins IX β IX γ and IX δ, are formed in the body besides the main pigment IX α(Z) by breaking the porphyrin rings in different positions (Fig 3*d* *e* and *f*) Still another isomer bilirubin IX α(E) (Fig 4) (Pedersen et al (37)) is probably a product of bilirubin transformation by phototherapy In all four of these isomers the carboxyl groups are shifted in relation to the other polar groups These substances therefore do not form the intramolecular hydrogen bonds which are present in bilirubin IX-α(Z) and are consequently soluble in water even in neutral or acid media

Soluble bilirubin isomers are excreted as such in bile or urine (25) whilst the slightly soluble bilirubin IX-α(Z) which accounts for the major part of excreted bile pigment is conjugated with glucuronic acid glucose or xylose forming soluble derivatives (24) The conjugation–excretion function of the liver is incompletely developed in the neonate so that bilirubin IX α(Z) accumulates during the first few days of life resulting in physiological neonatal jaundice

In summary the following bilirubins may be found in the human body IX α(Z) IX β IX γ IX δ, IX α(E), and conjugates of bilirubin IX α(Z) with glucuronic acid glucose and xylose Only one of these bilirubin IX α(Z) has a low solubility in water, due to the intra molecular hydrogen bonds, and only this compound is known to be neurotoxic The toxicity as outlined below probably depends upon precipitation of the insoluble acid form of this pigment in subcellular structures All the other substances are soluble and may presumably be regarded as non toxic (although experimental data on the toxicity of the newly discovered isomers are lacking)

Binding to albumin

Free bilirubin concentration

Bilirubin IX α(Z) is bound reversibly to one high affinity site on human serum albumin The binding constant at 37° is 7×10 mol/l (27 14) *Binding to two secondary sites seems* to play a minor role (14) If no other substances are bound to the same site it is possible to calculate the equilibrium concentration of free bilirubin IX α(Z) as

$$b_0=\frac{1}{7\times10^7}\times\frac{B}{A-B} \qquad (1)$$

where b_0 is the concentration of free bilirubin IX α(Z) in the absence of a competitor and A and B are the concentrations of albumin and bilirubin respectively In the average adult when $A=700$ μmol/l and $B=6$ μmol/l b_0 is $1\ 2\times10^{-10}$ mol/l or 0 12 nmol/l Considerably higher concentrations *of free bilirubin IX α(Z)* are found in icteric infants At a serum bilirubin concentration approaching the limit where exchange transfusion is considered when B is 310 μmol/l (18 mg/100 ml) and A

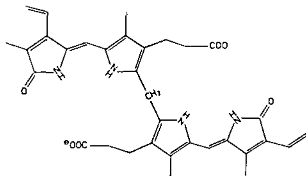


Fig 2 The anion of bilirubin IX-α(Z) which forms soluble salts in alkaline media

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The importance of pH

The risk of brain damage is increased in acidosis ((43) and references therein) Silberberg et al (39) in 1970 pointed out that this may be explained either by increased binding of bilirubin to the target or by decreased affinity of the binding to albumin Bilirubin binding to mitochondria to red blood cells and to fibroblasts in tissue culture is increased by lowering pH (7 34 35) while the bilirubin binding affinity to its first site on albumin is unchanged (29) Recalculation of the results of Brathd (7) for binding to erythrocytes shows that the binding constant is approximately proportional to the square of the hydrogen ion concentration Since bilirubin has two acid carboxyl groups with pK around 4 and shows no further acid dissociation even at a high pH (23) the binding of bilirubin to erythrocytes may be described as binding of the undissociated bilirubin acid with high affinity It seems likely that this mechanism is operative also at structures where the toxic effect is manifested

Binding to albumin occurs by an entirely different mechanism the divalent bilirubin anion is bound without participation of hydrogen ions and the affinity is therefore constant with varying pH This means that the safe limit of free bilirubin concentration varies with pH as described by the equation

$$\log \frac{\text{lim}\, b(\text{pH})}{50\ \text{nmol/l}} = 2(\text{pH} - 7\ 40) \qquad (2)$$

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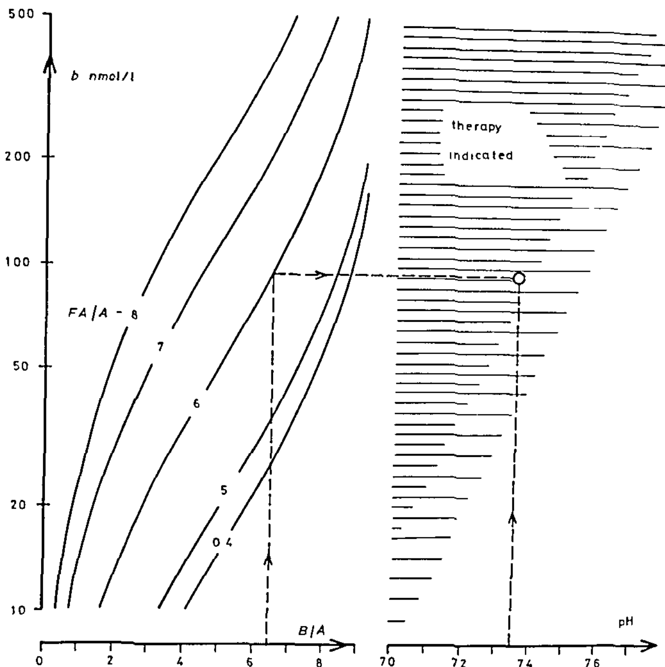


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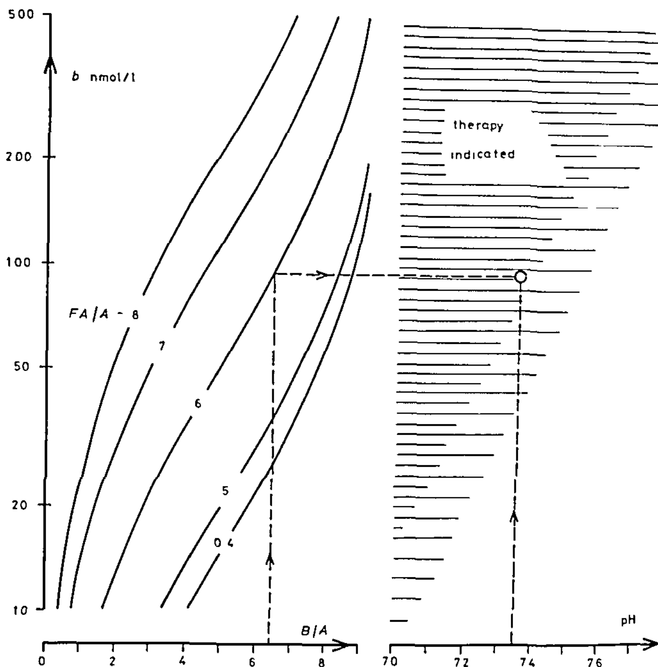


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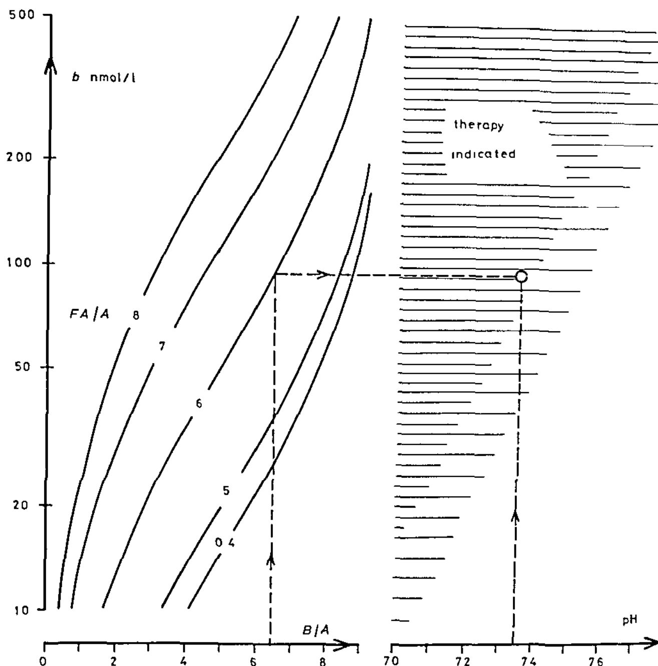


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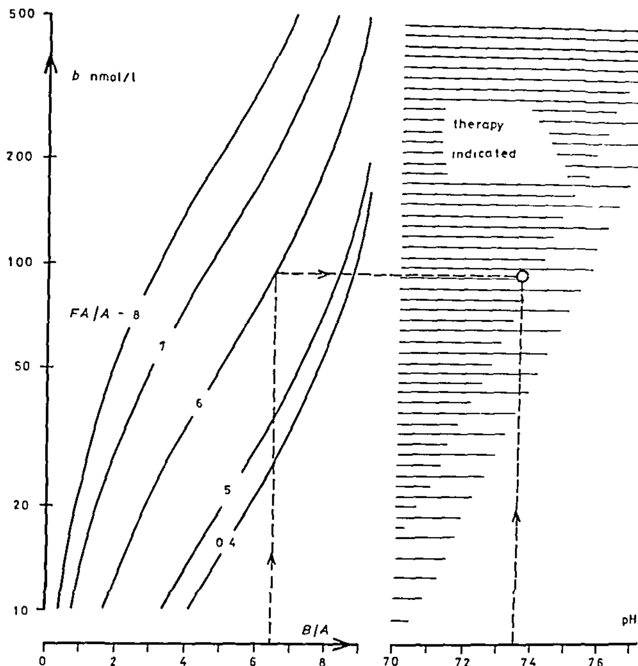


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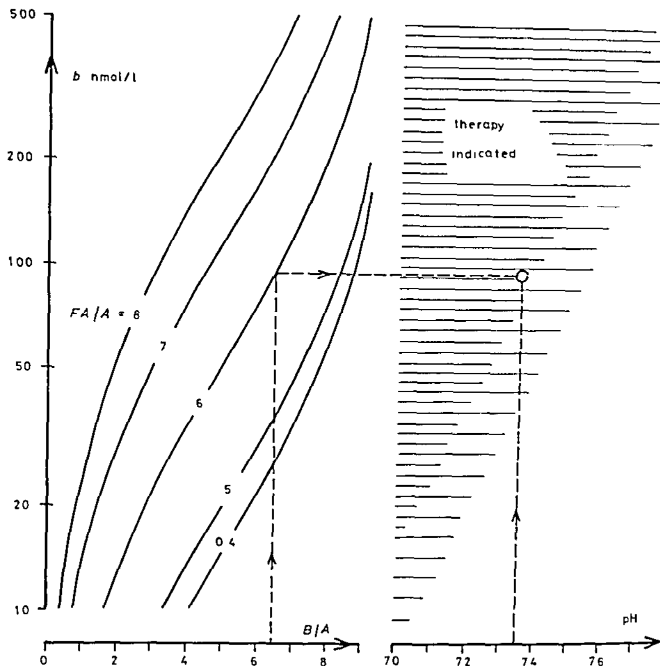


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carrying more bilirubin at a higher pH without risk of toxicity

In severe acidosis (plasma pH 7 05) the safe limit is thus 10 nmol/l whilst it is 100 nmol/l at pH 7 55 This is in good agreement with clinical experience and suggests that correc tion of plasma pH even to slightly above the normal level might be considered a fast and safe measure in the prevention of kernicterus The effect can again be measured by the bili rubin rebound An infant with a plasma pH 7 05 and a safe upper level of 10 nmol/l free bilirubin has plasma albumin less than half saturated with bilirubin (equation (1)) and has a bilirubin concentration about 150 to 200 μmol/l (9–12 mg/100 ml) After correction of pH the equilibrium is shifted in favour of the plasma albumin which can now carry a bili rubin concentration in the neighborhood of 300 to 400 μmol/l (18–24 mg/100 ml) and yet still have a tolerable level of the free pigment If an extracellular excess of bilirubin was pres ent before therapy success of the treatment should be signalled by an increase of plasma bilirubin concentration

Displacement of bilirubin by fatty acids

The amounts of long chain fatty acids nor mally present in plasma can be transported bound to albumin without interfering with bili rubin transport whilst fatty acid levels higher than four times the molar concentration of al bumin displace bilirubin from its primary bind ing site (41 47 20 44) Such fatty acid con centrations may be seen in certain disease states (21) and notably after parenteral nour ishment with fat emulsion (48) Andrew et al (1) using the Sephadex method for demon strating increased levels of free bilirubin have recommended that the molar ratio of fatty acid to albumin in the blood plasma be kept below 6 An empirical equation (14) derived from *in vitro* studies using the peroxidase technique can be used for estimation of the free bilirubin level during fatty acid loads i e when the fatty acid/albumin ratio is above 4 6

$$\frac{b}{b_0}=1\ 16(\mathrm{FA}/A-4\ 6)^{*}+1 \qquad (3)$$

where b is the free bilirubin concentration in the presence of fatty acid b_0 is calculated from equation (1), and FA is the molar con centration of long-chain fatty acids in the plasma It is seen that 6 mol fatty acid per mol albumin already appears to increase the free bilirubin concentration by a factor 2 6 while 5 mol seems to be permissible Final assessment of safe limits of fatty acid con centration must await an accepted method for determination of free bilirubin IX-α(Z) in un diluted plasma

A diagrammatic aid to indications for therapy

The above considerations are summarized in Fig 5 which comprises equations (1) (2) and (3) The diagram is used as follows Uncon jugated bilirubin albumin non esterified fatty acid and pH are measured in the infant s plasma (A small amount c 5% of bilirubin isomers and conjugates included in the bili rubin determination causes no serious error) The molar ratio of bilirubin to albumin B/A is calculated and entered on the abscissa in the left half of the diagram The fatty acid to al bumin molar ratio FA/A is used for selection of one of the curves The ordinate now indi cates the calculated concentration of free bili rubin IX α(Z) Keeping the same ordinate and entering the value of pH in the right half of the diagram leads to a point which may fall in the white the lightly shaded or the heavily shaded area A point in the white area shows that the plasma bilirubin concentration at the actual values of albumin fatty acid and pH does not indicate treatment A suggestive in dication exists in the intermediate area and a definite indication for therapy exchange transfusion albumin or alkali is present if the point falls in the dark upper left triangle An example (stippled lines) illustrates the pro- cedure

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sent free bilirubin IX α(Z) concentrations of 50 and 100 nmol/l respectively at pH 7 40 These limits are provisional and must be fixed by clinical experience and comparison with other systems of criteria for exchange trans fusion

The proposed method has limitations in be ing unable to take into account the effects of displacing drugs or of endogenous displac ing substances as well as other factors such as prematurity a history of previous anoxia etc

Displacing drugs

Certain sulfonamides and a number of other drugs can competitively displace bilirubin from its binding site on albumin thus increas ing the risk of kernicterus Clinical experience has shown that sulfisoxazole is capable of precipitating kernicterus in icteric infants (40) As pointed out by Stern (42) all drugs used in newborns or in pregnant or lactating women should be examined for bilirubin displacing effect This effect should be expressed in quantitative terms since many drugs are weak displacers (12 9) and some exert considerable displacement when given in large doses while being harmless in smaller amounts

The bilirubin displacement can be calcu lated from the equation

$$\frac{b}{b_0} = K_D d + 1 \quad (4)$$

where b is the free bilirubin IX-α(Z) concen tration in the presence of a displacing drug b_0 is calculated from equation (1) K_D is the binding constant of the drug to the bilirubin site on the albumin molecule and d is the free drug concentration in the plasma K_D is a con stant characteristic for each competitive drug which can be determined experimentally by the peroxidase technique (8) or by a double equilibrium dialysis against albumin and against albumin with one mol of bound bili rubin (13) Twenty sulfonamides have been examined by both methods Good agreement was obtained confirming the validity of either technique The peroxidase method is by far the easier and about two hundred substances mostly drugs have been tested In summary the results are as follows

The *sulfonamides* (13) show widely differing displacing effects ranging from strong dis placement with sulfaphenazole and sulfisoxa zole to a slight competition seen with sulfadia zine The latter compound appears safe at usual plasma levels and would be the drug of choice if a sulfonamide is given to an icteric newborn

Several *analgesic and antiinflammatory substances* are bilirubin displacers Some of these including salicylate (8 11 26) na proxen and phenylbutazone (12) have little effect at usual plasma levels but displace sig nificantly at higher concentrations Further studies with this group of substances are in dicated

Dicoumarol showed strong displacement in preliminary experiments Detailed work is needed with this and other anticoagulants

The strongest displacement was encoun tered with *X ray contrast media for cholangio graphy* These are used at very high plasma concentrations and are tailored to bind to al bumin in order to avoid excretion in the urine Binding includes the bilirubin site

Other compounds including the *benzodia zepines* are bound strongly to albumin but at a different site so they do not displace bili rubin (17)

The displacing effect of *stabilizers* in inject able albumin solutions (16) has been men tioned above Also the *parabenes* often used as antibacterial preservatives show significant displacement (8 38) These substances are used in large amounts as *food additives* so also is saccharin another bilirubin displacer Special attention is indicated if food additives are present in milk preparations used during the first few days of life and additives in the food of pregnant and lactating women should also be considered Although kernicterus has not been ascribed to these substances the possibility of minor brain damage should be

carrying more bilirubin at a higher pH without risk of toxicity

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A certain reservation is necessary when applying these *in vitro* results to clinical practice. Metabolites of drugs may displace more strongly than the compounds administered. This has been found with sulfonamides (13) which are metabolized predominantly by *N*(4) acetylation in the newborn infant. Seven *N*(4) acetylated sulfonamides have been tested and all these showed displacing effects equal to or stronger than those of the mother compounds. A hydroxylated metabolite of phenylbutazone appears to be a very strong displacer (12). It would therefore be very useful if a test could be designed to assess the displacing effect of drugs in the human body. Studies in Gunn rats (2, 22, 33) may also give suggestive results, although it must be remembered that the pattern of drug metabolism and binding to albumin often shows species differences.

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In the case of suspected bilirubin displacement, whether caused by fatty acids, by endogenous displacers, or by competitive drugs, a rational therapy is intravenous administration of albumin. More binding sites are then provided for bilirubin as well as for the competitor and, in case of exchange transfusion, certain amounts of both substances are removed from the body.

It should be noted that the plasma bilirubin concentration before treatment is likely to be low in these cases. If 6 mol fatty acid is present per mol albumin, a free bilirubin concentration of 50 nmol/l is reached when half of the albumin is occupied by bilirubin, as seen from the diagram (Fig. 5). Similar figures are obtained after sulfisoxazole in usual dosage (8). At usual levels of albumin, this occurs at a bilirubin concentration about 200 to 250 μmol/l (12–15 mg/100 ml) and therapy is needed if the plasma bilirubin is above this. The indication for treatment, therefore, cannot be obtained by determination of plasma bilirubin alone and, when displacers other than fatty acids are present, also not by use of the diagram. A method for quantitative determination of free bilirubin IX α(Z) in undiluted blood plasma would be helpful. The result of this determination could be entered as the ordinate in the diagram (Fig. 5) and would, together with the plasma pH in the abscissa, contribute rationally to the clinician's decision.

ACKNOWLEDGEMENTS

Thanks are due to the members of our team, T. Færch, Chr. Jacobsen, J. Jacobsen, A. O. Pedersen and Hanne Thiessen, for valuable discussions and criticism of the manuscript and to Frede Nielsen for graphical work.

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kept in mind, since food additives are given to large populations sometimes in considerable amounts and usually without declaration

The majority of drugs tested showed little or no displacing effect Most *antibiotics* (with the possible exception of some penicillin de rivatives) *antihistamines diuretics* and *general anaesthetics* so far investigated proved harmless All *drugs carrying a positive net charge* were found not to be displacing

Many drugs are given in small amounts, such as *hormones* some of the *synthetic diuretics digitoxin* etc and these will not displace bilirubin significantly even if they are bound strongly to the bilirubin site because the molar concentration available in the blood plasma is very small compared to that of albumin It is unnecessary to examine the displacing effect of such compounds

A certain reservation is necessary when applying these *in vitro* results to clinical practice Metabolites of drugs may displace more strongly than the compounds administered This has been found with sulfonamides (13), which are metabolized predominantly by $N(4)$ acetylation in the newborn infant Seven $N(4)$ acetylated sulfonamides have been tested and all these showed displacing effects equal to or stronger than those of the mother compounds A hydroxylated metabolite of phenylbutazone appears to be a very strong displacer (12) It would therefore be very useful if a test could be designed to assess the displacing effect of drugs in the human body Studies in Gunn rats (2 22 33) may also give suggestive results although it must be remembered that the pattern of drug metabolism and binding to albumin often shows species differences

Albumin therapy against bilirubin displacement

In the case of suspected bilirubin displacement whether caused by fatty acids by endogenous displacers or by competitive drugs a rational therapy is intravenous administration of albumin More binding sites are then provided for bilirubin as well as for the competitor and in case of exchange transfusion certain amounts of both substances are removed from the body

It should be noted that the plasma bilirubin concentration before treatment is likely to be low in these cases If 6 mol fatty acid is present per mol albumin a free bilirubin concentration of 50 nmol/l is reached when half of the albumin is occupied by bilirubin as seen from the diagram (Fig 5) Similar figures are obtained after sulfisoxazole in usual dosage (8) At usual levels of albumin this occurs at a bilirubin concentration about 200 to 250 μmol/l (12–15 mg/100 ml) and therapy is needed if the plasma bilirubin is above this The indication for treatment therefore can not be obtained by determination of plasma bilirubin alone and when displacers other than fatty acids are present also not by use of the diagram A method for quantitative determination of free bilirubin IX α(Z) in undiluted blood plasma would be helpful The result of this determination could be entered as the ordinate in the diagram (Fig 5) and would together with the plasma pH in the abscissa contribute rationally to the clinician s decision

ACKNOWLEDGEMENTS

Thanks are due to the members of our team T Færch Chr Jacobsen J Jacobsen A O Pedersen and Hanne Thiessen for valuable discussions and criticism of the manuscript and to Frede Nielsen for graphical work

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Submitted Dec 10 1976
Accepted Jan 25 1977

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CASE REPORT

OCULODENTODIGITAL DYSPLASIA SYNDROME

Report of Four Cases

C J THODÉN S RYÖPPY and P KUITUNEN

From the Children s Hospital University of Helsinki and Children s Castle Helsinki Finland

ABSTRACT Thoden C J Ryoppy S and Kuitunen P (Children s Hospital University of Helsinki and Children s Castle Helsinki Finland) Oculodentodigital dysplasia syndrome Report of four cases Acta Paediatr Scand 66 635 1977 —Four cases of oculodentodigital dysplasia are reported Three cases are from the same family father and two daughters These three cases have the characteristics typical of this disorder narrow nose hypoplastic alae nasi microphthalmia defects of the teeth syndactyly of the IV and V fingers and skeletal anomalies The fourth case differs from the earlier reported cases he has all the typical findings of oculodentodigital dysplasia but in addition he shows features not previously reported namely exceptionally poor vision mental retardation *monilethrix* and pili annuli changes of the hair

KEY WORDS Oculodentodigital syndrome

Lohman (8) was the first to describe a syndrome consisting of a typical face with hypoplastic alae nasi narrow nostrils microphthalmia microcornea anomalies of the iris and of syndactyly and camptodactyly of the fourth and fifth fingers and toes and enamel defects of the teeth Meyer Schwickerath et al (9) called the syndrome oculodentodigital dysplasia a name which later has been widely accepted in the literature Up to now 46 cases in 23 families have been published (1–16) Because no previous cases of oculodentodigital dysplasia have been reported from the Scandinavian countries we present here four cases which have been diagnosed at the Children s Hospital University of Helsinki and at the Children s Castle Helsinki Because of the similarities of signs and symptoms in cases 1 2 and 3 we report only cases 1 and 4 in more detail A summary of all four cases is given in Table 1

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Case 1 I T born 8 6 70 was studied at the age of four years She is a daughter of case 3 and the sister of case

There are no children in the family other than cases 1 and 2 (Fig 1a–c) The patient was born at term after an uneventful gestation with a birth weight of 3 300 g At the age of five months the girl was first seen at the Children s Hospital in Helsinki She had a peculiar face and nose the alae nasi were hypoplastic and the nostrils were narrow The patient had a high arched palate the eyes were small and the ears were prominent There was syndactyly of the fourth and fifth fingers on both hands The roentgenographs of the skull and the thorax were normal The ECG was normal as was the chromosomal analysis Ophthalmologic examination was performed at the age of four years The diameter of the cornea was 10 mm There were no anomalies of the iris or the lens According to the ophthalmologist the eye findings were normal The teeth were exceptionally worn and showed vertical streaks typical of amelogenesis imperfecta The X ray shadows of the primary teeth were faint the permanent teeth appeared normal The psychological examination revealed normal intelligence

Case 4 M R born 23 3 72 a boy was studied when two years old He is the oldest of three siblings The two younger siblings are healthy The patient was born at 38 weeks of gestation The birth weight was 3 740 g and

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Fig 2 Face of case 4

resent typical cases of oculodentodigital dysplasia which do not differ from cases reported elsewhere Case 4 with congenital blindness and retardation is an extremely severe variant of this syndrome All our patients have faces typical of oculodentodigital dysplasia a narrow nose and nostrils as well as hypoplastic alae nasi According to the literature the diameter of the cornea is between 6 and 10 5 mm (4 5 8 9 10 11 13 14) The corneal diameters of cases 1 2 3 and 4 were 10 8 5 9 and 8 mm The normal diameter of the adult cornea is said to be between 10 5 and 11 5 mm The retinal vessels of patient 4 were thread like—a finding not previously reported although a number of different eve anomalies have been observed (1 2 3) In a few reports it is stated that the patients had hypotrichosis or dry lusterless hair (4 5 6 7 9 14) which grew slowly In case 4 the anomaly of the hair could be exactly defined as a monilethrix change (alterations in the width of the hair) and as pili annuli (hair with spindle like air filled areas filling the whole marrow channel) Enamel defects of the teeth have been previously described (2 3 4 5 9 10 11 12 13 14) and were also found in our patients On the other hand our pediatric patients did not have broad mandibulae and alveolar ridges as has been reported in many cases (2 6 10 13) Our adult patient had broadening of the epiphyses of the long bones (4 7 13) and hyperostosis of the skull bones (13) In our two older children we could see only a slight broadening of the long bones The patients had the typical changes of the hands and feet (Table 1 Fig 3*a b* 1–15) Therapeutically little can be done except for the surgical correction of the syndactylies Because of the osseous anomalies this should be done early and even then a perfect result is not easily obtained The pa

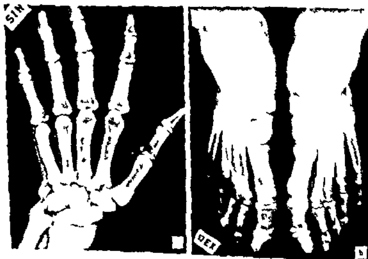


Fig 3 (*a*) Hand of case 3 Note cube shaped middle phalanx in fifth finger (*b*) Foot of case 3 Note absence of middle phalanges of toes

Table 1 *Main findings in four cases of oculodentodigital dysplasia*

	Case 1	Case 2	Case 3	Case 4
Age	I T 4 years	P T 3 years	A T 28 years	M R 2 years
Sex	Female	Female	Male	Male
Typical face	+	+	+	+
Syndactyly of IV and V fingers	+	+	+	+
Skeletal findings				
Aplasia of middle phal of toes II–V	+	+	+	+
Hypoplasia of middle phal of finger V	+	+	+	(+)[a]
Broadening of the long bones	–	–	+	–
Hyperostosis of skull bones	–	–	+	–
Dental findings				
Enamel defects	+	+	+	+
Poor mineralisation	+	+	+	+
Eye findings				
Diameter of the cornea	10 mm	8 5 mm	9 mm	8 mm
Poor vision	–	–	–	+
Abnormal eye fundi	–	–	–	+
Hair	Normal	Normal	Normal	Abnormal[b]
Intelligence	Normal	Normal	Normal	Subnormal

[a] Aplasia of middle phal of finger V
[b] Monilethrix and pili annuli changes

the Apgar score was 7 At the age of two days the boy was admitted at the Children s Hospital in Helsinki On admission the typical findings of oculodentodigital dysplasia as described in case 1 were found (Fig 2) In the ophthalmologic examination microphthalmia and microcornea were seen The diameter of the cornea was 8 mm at the age of 1 5 years The fundi of the eyes showed greyish papillae the vessels were narrow and thread like the retinae were pale The patient had nystagmus and he possibly perceived light The lower incisors appeared at the age of seven months all teeth were yellow and there was an enamel defect Audiometry showed a 40–50 dB loss of hearing which might have been a result of frequent middle ear infections The hair was coarse and grew poorly under the microscope monilethrix as well as the changes of pili annuli were found Pneumoencephalography at the age of 4 5 months revealed a slight central atrophy EEG was repeatedly normal The ECG was abnormal there was a low QRS complex and the T waves were isoelectric or negative but the patient had no symptoms or signs of cardiac disease The cause of the ECG findings thus remains obscure The chromosomal analysis showed a normal karyotype Mental development was slow At the age of two years the patient reacted to some familiar phrases He would reach for some desirable toy which he would then transfer from one hand to the other

DISCUSSION

The four cases reported here are as far as we know the first cases to be reported from the Scandinavian countries Cases 1 2 and 3 rep



Fig 1 (*a–c*) Faces of cases 1–3



Fig 2 Face of case 4

resent typical cases of oculodentodigital dysplasia which do not differ from cases reported elsewhere Case 4 with congenital blindness and retardation is an extremely severe variant of this syndrome All our patients have faces typical of oculodentodigital dysplasia a narrow nose and nostrils as well as hypoplastic alae nasi According to the literature the diameter of the cornea is between 6 and 10 5 mm (4 5 8 9 10 11 13 14) The corneal diameters of cases 1 2 3 and 4 were 10 8 5 9 and 8 mm The normal diameter of the adult cornea is said to be between 10 5 and 11 5 mm The retinal vessels of patient 4 were thread like—a finding not previously reported although a number of different eye anomalies have been observed (1 2 3) In a few reports it is stated that the patients had hypotrichosis or dry lusterless hair (4 5 6 7 9 14) which grew slowly In case 4 the anomaly of the hair could be exactly defined as a monilethrix change (alterations in the width of the hair) and as pili annuli (hair with spindle like air filled areas filling the whole marrow channel) Enamel defects of the teeth have been previously described (2 3 4 5 9 10 11 12 13 14) and were also found in our patients On the other hand our pediatric patients did not have broad mandibulae and alveolar ridges as has been reported in many cases (2 6 10 13) Our adult patient had broadening of the epiphyses of the long bones (4 7 13) and hyperostosis of the skull bones (13) In our two older children we could see only a slight broadening of the long bones The patients had the typical changes of the hands and feet (Table 1 Fig 3*a b* 1–15) Therapeutically little can be done except for the surgical correction of the syndactylies Because of the osseous anomalies this should be done early and even then a perfect result is not easily obtained The pi

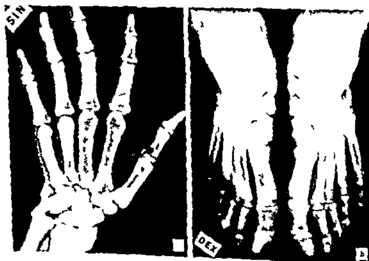


Fig 3 (*a*) Hand of case 3 Note cube shaped middle phalanx in fifth finger (*b*) Foot of case 3 Note absence of middle phalanges of toes

Table 1 *Main findings in four cases of oculodentodigital dysplasia*

	Case 1	Case 2	Case 3	Case 4
Age	*I T 4 years*	*P T 3 years*	*A T 28 years*	*M R 2 years*
Sex	Female	Female	Male	Male
Typical face	+	+	+	+
Syndactyly of IV and V fingers	+	+	+	+
Skeletal findings				
Aplasia of middle phal of toes II–V	+	+	+	+
Hypoplasia of middle phal of finger V	+	+	+	()
Broadening of the long bones	–	–	+	–
Hyperostosis of skull bones	–	–	+	–
Dental findings				
Enamel defects	+	+	+	+
Poor mineralisation	+	+	+	+
Eye findings				
Diameter of the cornea	10 mm	8 5 mm	9 mm	8 mm
Poor vision	–	–	–	+
Abnormal eye fundi	–	–	–	+
Hair	Normal	Normal	Normal	Abnormal[b]
Intelligence	Normal	Normal	Normal	Subnormal

Aplasia of middle phal of finger V
[b] Monilethrix and pili annuli changes

the Apgar score was 7 At the age of two days the boy was admitted at the Children s Hospital in Helsinki On admission the typical findings of oculodentodigital dysplasia as described in case I were found (Fig 2) In the ophthalmologic examination microphthalmia and microcornea were seen The diameter of the cornea was 8 mm at the age of 1 5 years The fundi of the eyes showed greyish papillae the vessels were narrow and thread like the retinae were pale The patient had nystagmus and he possibly perceived light The lower incisors appeared at *the age of seven months all teeth were yellow and there* was an enamel defect Audiometry showed a 40–50 dB loss of hearing which might have been a result of frequent middle ear infections The hair was coarse and grew poorly under the microscope monilethrix as well as the changes of pili annuli were found Pneumoencephalography at the age of 4 5 months revealed a slight central atrophy EEG was repeatedly normal The ECG was abnormal there was a low QRS complex and the T waves were isoelectric or negative but the patient had no symptoms or signs of cardiac disease The cause of the ECG findings thus remains obscure The chromosomal analysis showed a normal karyotype Mental development was slow At the age of two years the patient reacted to some familiar phrases He would reach for some desirable toy which he would then transfer from one hand to the other

DISCUSSION

The four cases reported here are as far as we know the first cases to be reported from the Scandinavian countries *Cases 1 2 and 3 rep*



Fig 1 (*a–c*) Faces of cases 1–3



Fig 2 Face of case 4

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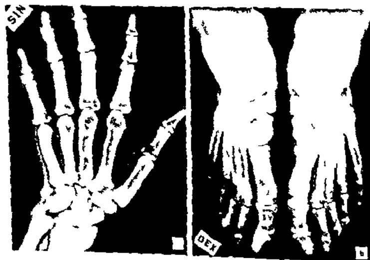


Fig 3 (*a*) Hand of case 3 Note cube shaped middle phalanx in fifth finger (*b*) Foot of case 3 Note absence of middle phalanges of toes

tients with oculodentodigital dysplasia have usually been of normal intelligence, only a few slightly retarded patients (14) have been re ported In this respect case 4 represents an exception with slow intellectual and physical development This could be either explained as a very severe case of oculodentodigital dysplasia or the patient could have suffered from an intrauterine or a perinatal injury The reason for the central atrophy found in the pneumoencephalogram cannot be explained satisfactorily with the available facts Our pa tients represent both types of inheritance known to occur in oculodentodigital dysplasia (13) Cases 1–3 seem to be dominantly in herited case 4 represents the sporadically oc curring type of oculodentodigital dysplasia

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Submitted Oct 18 1976
Accepted Jan 27 1977

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CASE REPORT

NEONATAL THYROTOXICOSIS

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ABSTRACT Petersen S and Serup J (Department of Paediatrics and Department of Obstetrics and Gynaecology Central Hospital Nykøbing Falster Denmark) Neonatal thyrotoxicosis Acta Paediatr Scand 66 639 1977.—Neonatal thyrotoxicosis is a transient hyperthyroidism in infants of mothers with current or previous thyrotoxicosis The pathogenesis has been accepted to be placental transfer of maternal thyroid stimulating immunoglobulins Of two siblings from a previous thyrotoxic mother the first had marked symptoms of thyrotoxicosis but during the second pregnancy antithyroid treatment was given to the mother and though the child had high levels of thyroid hormones for 6 weeks it had only minimal symptoms of thyrotoxicosis

KEY WORDS Hyperthyroidism newborn long acting thyroid stimulator human thyroid stimulating immunoglobulin

A transient hyperthyroid condition may be seen in newborn infants of mothers with previous or current thyrotoxicosis Cardiac failure is responsible for a lethality of about 16% in the previously reported cases (6) Placental transfer of long acting thyroid stimulator (LATS) is considered responsible for a number of cases but recently another immunoglobulin human thyroid stimulating immunoglobulin (HTSI) has been detected in some cases Antithyroid treatment of the mother during pregnancy is important in preventing serious disease in the newborn This is a report of 2 siblings who demonstrated a striking difference between the untreated and treated course of the disease

METHODS

Total serum thyroxine concentration (T) was assayed using the Thyopac® 5 kit from The Radiochemical Centre Amersham England Normal ranges were 65–145 nmol/l in adults and 65–175 nmol/l in pregnant women

Free thyroxine (FT_4) was assayed as normalized thyroxine ratio by the same Thyopac® 5 kit Normal ranges in adults and in pregnant women were 0 88–1 11 relative units

Serum tri iodothyronine (T_3) was assayed using The Radiochemical Centre s T_3 RIA kit Normal ranges were 1 3–3 5 nmol/l in adults and 1 5–5 0 nmol/l in pregnant women

Long acting thyroid stimulator (LATS) was assayed using white guinea pigs (4) In this technique LATS levels below 1 25 rel U were normal 1 25–1 50 was suspected elevated and more than 1 50 was considered significant pathologic

CASE REPORTS

The mother had exophthalmic hyperthyroidism at the age of 17 years She had undergone partial thyroidectomy and had since been clinically euthyroid without further treatment A mild exophthalmos remained Non pregnant laboratory data T_4 155 nmol/l and FT_4 1 13 rel U

Case 1

Her first child a girl was delivered 7½ years after the thyroidectomy by a Caesarean section at 34 weeks gestation because of prolapsed umbilical cord Birth weight was 2050 g and Apgar score 10 She was dysmature with a slight exophthalmos and thyroid enlargement The in

tients with oculodentodigital dysplasia have usually been of normal intelligence only a few slightly retarded patients (14) have been reported In this respect case 4 represents an exception with slow intellectual and physical development This could be either explained as a very severe case of oculodentodigital dysplasia or the patient could have suffered from an intrauterine or a perinatal injury The reason for the central atrophy found in the pneumoencephalogram cannot be explained satisfactorily with the available facts Our patients represent both types of inheritance known to occur in oculodentodigital dysplasia (13) Cases 1–3 seem to be dominantly inherited, case 4 represents the sporadically occurring type of oculodentodigital dysplasia

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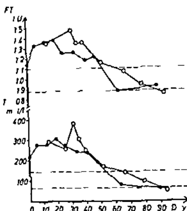


Fig 3 Serum thyroxine (T) and free thyroxine (FT_4) levels in case 1 O—O and case 2 ●—● Normal range in adults = = =

In cord blood T_4 was [illegible] nmol/l and FT was [illegible] rel U T_3 from 2–4 weeks was more than 9 nmol/l and at 5 weeks it decreased to 7 7 nmol/l LATS in cord blood was 0 81 rel U at 4 weeks it was 0 97 rel U and at 6 weeks it was [illegible] rel U Chest X ray was normal and bone age at 3 months was 1 year 3 months

DISCUSSION

White (13) presented in 1912 the first description of neonatal thyrotoxicosis In 1958 Adams (1) detected the long acting thyroid stimulator (LATS) and when McKenzie (7) in 1964 presented five cases of neonatal thyrotoxicosis in which LATS was present in mother and child it seemed reasonable to believe that LATS which is an immunoglobulin G and is able to pass the placenta was responsible for the disease in the newborn Several cases in which LATS was detected have been published but in some recent cases it was impossible to determine LATS in the mothers and in the children (14) In 1974 Nutt et al (9) presented a case in which no LATS was found They found another immunoglobulin LATS protector (LATS P) later called human thyroid stimulating immunoglobulin (HTSI) in mother and child The importance of HTSI in LATS negative cases of neonatal thyrotoxicosis has been confirmed by Dirmikis & Munro (2) and Mitchell et al (8)

Thyrotoxic symptoms in the newborn appear within the first week of life but in cases where the mother has been treated during pregnancy the symptoms may be vague delayed or fail to appear Preterm birth restlessness low weight gain diarrhoea tachycardia thyroid enlargement and exophthalmos are common findings Cardiac failure is a frequent and serious complication Accelerated bone maturity is common Rare findings are thymic enlargement and idiopathic thrombocytopenic purpura (6) The disappearance of the symptoms has been related to the elimination of the passively transferred thyroid stimulating immunoglobulins

From about 20 weeks gestation the endocrine regulatory function of the foetus is fully developed From this time it may be possible for the thyroid stimulating immunoglobulins to produce intrauterine thyrotoxicosis which may appear with foetal tachycardia and active movements Antithyroid therapy should be given to the mother from this time to render the foetus euthyroid and to reduce the risk of preterm birth The treatment will clearly reduce the severity of thyrotoxicosis in the newborn This can be due to reduction of the immunoglobulin level (4) but it can also be an effect of placentally transferred antithyroid drugs

The different clinical symptoms in our 2 siblings the first untreated and the second treated during pregnancy confirms the effect of this treatment The laboratory findings were almost identical in the two infants and it is unclear why case 2 was not clinically thyrotoxic This may be a result of the peripheral effect of transplacental transferred propylthiouracil which blocks the extrathyroid conversion of T_4 to the biologically more active T_3 (5)

To prevent neonatal hyperthyroidism treatment should be given to the mother during pregnancy and in case of previous or current thyrotoxicosis of the mother investigation for LATS and HTSI is important in predicting neonatal thyrotoxicosis

If a newborn becomes thyrotoxic the treat



Fig 1 Case 1 Exophthalmos 3 weeks old

fant was restless and had diarrhoea During the second week of life exophthalmos (Fig 1) thyroid enlargement (Fig 2) and hyperactivity progressed Heart rate was 180–200 per min but there was no heart murmur or enlarged liver She was treated with phenobarbital and supplementary oxygen in an incubator From the age of 4 weeks the symptoms subsided and from 6 weeks regression in exophthalmos and thyroid enlargement was observed The weight gain was slow for the first 8 weeks

The T_4 and FT_4 levels are seen in Fig 3 The T_3 level was 6 5 nmol/l at 6 weeks and 1 6 nmol/l at 12 weeks Quantitative estimation of LATS was not available for us at that time but qualitatively some LATS activity was detected at 5 weeks in mother and infant Chest X ray showed extreme thymic enlargement At 3 months the bone age according to Greulich & Pyle was 1½–2 years There was no signs of cranial synostosis At 2½ years of age the development was normal She was euthyroid with normal T_4 and FT_4

Case 2

During her second pregnancy 2½ years after the first the mother was without any clinical signs of hyperthyroidism At 18 weeks gestation her T_4 was 264 nmol/l FT_4 1 22 rel U and T_3 7 0 nmol/l At 26 weeks gestation the foetal heart rate increased from 140 to 165 per min and the foetal movements were more active This was regarded as symptoms of foetal thyrotoxicosis and the mother was given antithyroid treatment with propylthiouracil 300 mg per day for 2 weeks and the dose was gradually decreased to a daily dose of 62 5 mg at 32 weeks gestation During treatment T_4 decreased to 130 nmol/l FT_4 to 0 95 rel U and T_3 to 5 9 nmol/l The foetal movements and heart rate returned to normal Propylthiouracil in a daily dose of 62 5 mg was continued until the day of delivery and the T_4 and FT_4 remained within normal limits The LATS investigation showed at 18 weeks 0 87 rel U at 26 weeks 1 56 rel U and at 34 weeks 0 76 rel U

The child a boy was born spontaneously at 37 weeks gestation He was asphyxiated with Apgar score 5 after 1 min and 10 after 10 min Birth weight was 2 500 g During the first day of life he was treated with supplementary oxygen in an incubator because of mild respiratory distress There was no exophthalmos or thyroid enlargement He was trembling somewhat during the first 3 days and was treated with phenobarbital The heart rate was 160 The weight gain was slow for the first 3 weeks probably because of maternal hypogalactia but after supplementary feeding with evaporated milk products he was gaining weight properly Though the levels of thyroid hormones were almost as high as in case 1 (Fig 3) and much exceeded the physiological increased levels in the neonatal period (10) he never showed clinical symptoms of thyrotoxicosis except a slight lid retraction



Fig 2 Case 1 Thyroid enlargement 4 weeks old

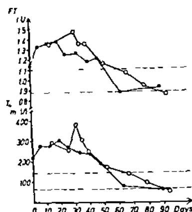


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If a newborn becomes thyrotoxic the treat

ment recommended is symptomatically pheno barbital and digoxin and antithyroid treatment with propylthiouracil and iodides (3) Propranolol is reported very effective (12)

The obstetric details of our cases are described elsewhere (11)

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Submitted Dec 2 1976
Accepted Febr 5 1977

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Acta Pædiatr Scand 66 643–647 1977

CASE REPORT

A CASE OF APPARENT HYPOPITUITARISM COMPLICATING CHRONIC INFLAMMATORY BOWEL DISEASE IN CHILDHOOD AND ADOLESCENCE

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ABSTRACT Green J R B O Donoghue D P Edwards C R W and Dawson A M (Departments of Gastroenterology and Medicine St Bartholomew s Hospital London England) A case of apparent hypopituitarism complicating chronic inflammatory bowel disease in childhood and adolescence Acta Paediatr Scand 66 643 1977.—There is conflicting evidence regarding the adequacy of hypothalamic pituitary function in children and adolescents with chronic inflammatory bowel disease complicated by growth retardation and delayed sexual maturation A child with Crohn s disease who has never received corticosteroid therapy had delay of both growth and sexual maturation and has been investigated over the course of his disease In addition to a skull X ray (normal) and thyroid function tests (normal) a standard insulin tolerance test (insulin 0 15 u/kg) and a standard gonadotrophin releasing hormone (Gn RH) test (100 μg Gn RH i/v) were performed when the bowel disease was in relapse and again during a remission of the bowel disease achieved by surgery When the bowel disease was in relapse (coincident with growth arrest) results showed an inadequate release of gonadotrophins and of growth hormone (even after pre treatment with stilboestrol) but normal release of cortisol and prolactin During a remission of the bowel disease coinciding with a period of rapid catch up growth release of growth hormone was normal and that of gonadotrophins supranormal The demonstration of a reversible apparent partial hypopituitarism in this boy not only re-questions the adequacy of hypothalamic pituitary function in inflammatory bowel disease but also indicates a potential diagnostic pitfall in the routine investigation of growth retardation if gastrointestinal symptoms are not prominent at presentation

KEY WORDS Crohn s disease growth retardation growth hormone partial hypopituitarism

Delayed skeletal growth and sexual maturation are recognised complications of chronic inflammatory bowel disease occurring in childhood or adolescence (1 7 15) If gastrointestinal symptoms are prominent at presentation the cause of the growth stunting may be obvious However difficulty in diagnosis may arise in cases where delayed maturation precedes gastrointestinal symptoms (13)

The pathogenesis of growth retardation in inflammatory bowel disease remains uncertain Previous studies have shown either impaired (8) or normal (3) stimulated growth hormone release in severely growth retarded children and adolescents with inflammatory bowel disease The latter finding of normal growth hormone release in these patients has subsequently been the more widely accepted

In view of this conflicting evidence the results of this detailed study of a boy with

ment recommended is symptomatically phenobarbital and digoxin and antithyroid treatment with propylthiouracil and iodides (3) Propranolol is reported very effective (12)

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Submitted Dec 2 1976
Accepted Febr 5 1977

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Acta Pædiatr Scand 66 643–647 1977

CASE REPORT

A CASE OF APPARENT HYPOPITUITARISM COMPLICATING CHRONIC INFLAMMATORY BOWEL DISEASE IN CHILDHOOD AND ADOLESCENCE

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ABSTRACT Green J R B O Donoghue D P Edwards C R W and Dawson A M (Departments of Gastroenterology and Medicine St Bartholomew s Hospital London England) A case of apparent hypopituitarism complicating chronic inflammatory bowel disease in childhood and adolescence Acta Paediatr Scand 66 643 1977 —There is conflicting evidence regarding the adequacy of hypothalamic pituitary function in children and adolescents with chronic inflammatory bowel disease complicated by growth retardation and delayed sexual maturation A child with Crohn s disease who has never received corticosteroid therapy had delay of both growth and sexual maturation and has been investigated over the course of his disease In addition to a skull X ray (normal) and thyroid function tests (normal) a standard insulin tolerance test (insulin 0 15 u/kg) and a standard gonadotrophin releasing hormone (Gn RH) test (100 μg Gn RH i/v) were performed when the bowel disease was in relapse and again during a remission of the bowel disease achieved by surgery When the bowel disease was in relapse (coincident with growth arrest) results showed an inadequate release of gonadotrophins and of growth hormone (even after pre treatment with stilboestrol) but normal release of cortisol and prolactin During a remission of the bowel disease coinciding with a period of rapid catch up growth release of growth hormone was normal and that of gonadotrophins supranormal The demonstration of a reversible apparent partial hypopituitarism in this boy not only re-questions the adequacy of hypothalamic pituitary function in inflammatory bowel disease but also indicates a potential diagnostic pitfall in the routine investigation of growth retardation if gastrointestinal symptoms are not prominent at presentation

KEY WORDS Crohn s disease growth retardation growth hormone partial hypopituitarism

Delayed skeletal growth and sexual maturation are recognised complications of chronic inflammatory bowel disease occurring in childhood or adolescence (1 7 15) If gastrointestinal symptoms are prominent at presentation the cause of the growth stunting may be obvious However difficulty in diagnosis may arise in cases where delayed maturation precedes gastrointestinal symptoms (13)

The pathogenesis of growth retardation in inflammatory bowel disease remains uncertain Previous studies have shown either impaired (8) or normal (3) stimulated growth hormone release in severely growth retarded children and adolescents with inflammatory bowel disease The latter finding of normal growth hormone release in these patients has subsequently been the more widely accepted

In view of this conflicting evidence the results of this detailed study of a boy with

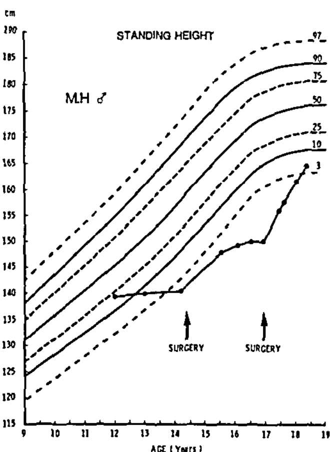


Fig 1 Standing height record of M H between the ages of *11 5* years and *18* years Arrows indicate the timing of the two laparotomies (Chart from Tanner 1958 [14])

Crohn s disease and severe growth retardation have two implications firstly they re open the question of the adequacy of growth hormone release in this condition and secondly they *demonstrate a potential diagnostic pitfall in the* routine investigation of growth retardation

CASE HISTORY

This boy first presented at the referring hospital when 12 years old with a nine month history of morning nausea occasional vomiting and intermittent colicky upper abdominal pain Physical examination was normal and he was just below the 25th percentile for both height and weight Investigations including a barium meal and follow through examination were normal A diagnosis of periodic syndrome was made and a low milk low fat diet was prescribed initially with improvement of his symptoms

Two years later when aged 14 2 years he was again referred to the same hospital with an eighteen month history of recurrent intermittent upper abdominal colicky pain occasional vomiting and failure to grow Physical examination was again normal except that he was now below the 3rd percentile for height and weight Barium follow through examination now showed extensive small bowel abnormalities consistent with Crohn s disease At subsequent laparotomy 150 cm of involved ileum and jejunum were resected Histology confirmed Crohn s disease and the patient made a satisfactory post-operative recovery on no treatment He remained symptom free for just one year and his height increased by 7 cm although he still remained below the 3rd percentile (Fig 1)

At age 15 5 years he developed a recurrence of nausea colicky upper abdominal pain and episodic abdominal distension Barium follow through examination showed changes of recurrent Crohn s disease particularly affecting the jejunum Eventually after a trial of salazopyrine the patient was referred for further management

He was first admitted to this hospital for investigation at the age of 16 9 years At that time he continued to complain of the same abdominal symptoms but had no diarrhoea Abdominal examination sigmoidoscopy and rectal biopsy were all normal as was the barium enema examination His height was now well below the 3rd percentile (as was his weight) and he was between stages 3 and 4 for both pubic hair and genital development with testes of 12 and 15 ml sparse axillary hair and slight voice deepening Skull X rays were normal and bone age was 13 1 years Thyroid function was normal with a free thyroxine index of 5 9 (normal range 4 5–13 5) Plasma gonadotrophins after 100 μg of intravenous gonadotrophin releasing hormone (Gn RH) showed an impaired pubertal type of response (10) (Table 1) After intravenous soluble insulin (0 15 u/kg) adequate hypoglycaemia was obtained but the plasma growth hormone response was impaired (4) (Table 2) This was not improved by two days of pretreatment with stilboestrol 1 mg tds (2 6) (Table 2) Plasma cortisol response (717 nmol/l) and plasma prolactin response (15 μg/l) to hypoglycaemia were normal (Table 2)

At subsequent laparotomy a further 90 cm of jejunum was removed and post-operatively the patient was started on azathioprine (2 mg/kg) Six months post-operatively with symptoms in full remission the fasting plasma growth hormone was much higher than on both previous occasions and values of over 20 μg/l were recorded after adequate hypoglycaemic stimulus (Table 2) the accepted criterion of a normal response (4) Basal gonadotrophin

Table 1 *Gonadotrophin releasing hormone (Gn RH) tests in relapse and remission (M H)*

Gn RH 100 μg i v given at 0 min Gonadotrophin assay standards FSH-MRC 68/39 LH-MRC 68/40

	Relapse		Remission	
Time (min)	FSH (u/l)	LH (u/l)	FSH (u/l)	LH (u/l)
0	<0 2	<0 4	6 3	13 9
20	1 2	1 9	11 5	>60
60	1 7	2 4	13 5	>60

Table 2 *Insulin tolerance tests in relapse and remission (M H)*

Soluble insulin 0 15 u/kg i v given at 0 min Blood sugar concentration <1 8 mmol at 30 min on each occasion with marked sweating

	Relapse				Remission
	Growth hormone (μg/l)				
Time (min)	Pre stilboestrol	Post stilboestrol	Prolactin (μg/l)	Cortisol (nmol/l)	Growth hormone (μg/l)
0	8 7	5	<4	303	19
30	8	7 4	5	303	22
45	11 9	10 3	14	634	17
60	8 9	7 6	15	717	17
90	9 7	4 2	10	510	19
1 0	5 9	5 6	<4	359	21

concentrations were now elevated with an exaggerated response to Gn RH (Table 1) One year post-operatively aged 18 years he remains asymptomatic He is now sexu ally mature and his height (164 cm) is approaching the tenth percentile (Fig 1) His bone age (16 5 years) more nearly matches his chronological age

The patient has never received corticosteroid therapy at any stage of his illness

DISCUSSION

There are few studies of growth hormone sta tus in children and adolescents with inflamma tory bowel disease complicated by delayed growth and sexual maturation In the largest single study McCaffery et al (8) reported that 11 of 13 severely growth retarded children with inflammatory bowel disease failed to achieve adequate plasma growth hormone concentrations after hypoglycaemic stimulus This study could be criticised however in that all but two patients had previously re ceived significant corticosteroid therapy and repeat stimulation after stilboestrol pre treat ment was not performed Furthermore there was no indication of whether or not growth hormone responses returned to normal after a remission of the bowel disease had been achieved In contrast Gotlin & Dubois (3) found normal nyctohemeral plasma growth hormone concentrations and normal plasma growth hormone response after arginine in fusion in 6 severely growth retarded adoles cents with inflammatory bowel disease This together with the objections to the previous study has led to the prevalent view that growth hormone release is not impaired in pa tients with growth retardation and inflamma tory bowel disease In view of this conflict of evidence the patient we report here provided a rare opportunity for endocrinological study as he has never received corticosteroid therapy

The present study shows that when the pa tient s disease was in relapse his stimulated growth hormone release was inadequate even after stilboestrol pre treatment Impaired growth hormone release may occasionally be found in some normal subjects undergoing puberty but pre treatment with stilboestrol is found to correct this to normal (2 6) In contrast when he was in clinical remission both fasting plasma growth hormone concen tration and the response to hypoglycaemia were well within normal limits A parallel change was seen in the plasma gonadotrophin response to Gn RH The cause of the exag gerated response to Gn RH found during a re mission of the bowel disease is uncertain it may relate either to a de suppression effect associated with remission of the bowel disease or possibly may be due to therapy with aza thioprine at the time of re testing In contrast both plasma cortisol and plasma prolactin re sponses to hypoglycaemia remained normal throughout

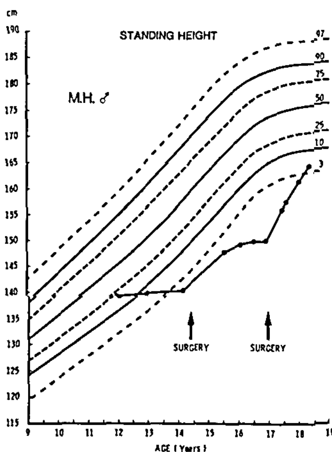


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The present study shows that when the patient s disease was in relapse his stimulated growth hormone release was inadequate even after stilboestrol pre treatment Impaired growth hormone release may occasionally be found in some normal subjects undergoing puberty but pre treatment with stilboestrol is found to correct this to normal (2 6) In contrast when he was in clinical remission both fasting plasma growth hormone concentration and the response to hypoglycaemia were well within normal limits A parallel change was seen in the plasma *gonadotrophin* response to Gn RH The cause of the exaggerated response to Gn RH found during a remission of the bowel disease is *uncertain it* may relate either to a de suppression effect associated with remission of the bowel disease or possibly may be due to therapy with azathioprine at the time of re testing In contrast both plasma cortisol and plasma prolactin responses to hypoglycaemia *remained* normal throughout

The conclusion from these investigations is that, when clinical disease was in relapse the pattern of endocrine findings was indistinguishable from that seen in partial hypopituitarism These abnormalities were transient because they disappeared when the bowel disease was in remission Findings similar to these have been reported in growth retardation due to coeliac disease (16) and severe emotional deprivation (12)

It is difficult to reconcile the results of this boy with the normal arginine provocation test results of Gotlin & Dubois (3) In view of this conflict caution should perhaps be exercised before excluding the possibility of an abnormality of growth hormone release in this condition as the differential response of the pituitary to the two stimuli may indicate a more subtle disturbance of function Whatever the role of pituitary growth hormone release in this condition, there may also be an additional element of end organ resistance to growth hormone as McCaffery and colleagues (9) have shown in a small and rather unsatisfactory study that administration of human growth hormone to three stunted patients with inflammatory bowel disease produced no dramatic increase in height

Malnutrition from a combination of anorexia malabsorption and increased enteric protein loss no doubt contributes directly to the growth retardation of patients with inflammatory bowel disease However prolonged parenteral nutrition (without surgery) has been found to lead only to a partial restoration of linear height (5) so nutritional status is therefore only one of probably many factors in the retardation of growth That malnutrition might be more directly responsible for the growth hormone abnormalities demonstrated in this boy seems unlikely since children with gross protein–calorie malnutrition (marasmus) and growth retardation have very high basal plasma growth hormone concentrations (11)

In conclusion this boy with Crohn s disease and growth retardation had an impairment of stimulated pituitary release of both plasma growth hormone and plasma gonadotrophins when the bowel disease was in relapse These apparent defects disappeared after a remission of the Crohn s disease had been achieved by surgery These results therefore re question the normality of pituitary function previously accepted in such patients Furthermore since both insulin induced hypoglycaemia and the Gn RH test are widely used in the routine investigation of growth retardation these results demonstrate how patients with occult inflammatory bowel disease may be wrongly diagnosed as having partial hypopituitarism unless the possibility of underlying inflammatory bowel disease is specifically considered

ACKNOWLEDGMENTS

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Submitted Dec 8 1976
Accepted March 16 1977

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Acta Pædiatr Scand 66 649–651 1977

CASE REPORT

ANEURYSM OF THE NONPATENT DUCTUS ARTERIOSUS IN THE NEWBORN

M RUTISHAUSER G RONEN and F WYLER

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ABSTRACT Rutishauser M Ronen G and Wyler F (Department of Paediatrics University Children s hospital Basel Switzerland) Aneurysm of the nonpatent ductus arteriosus in the newborn Acta Paediatr Scand 66 649 1977.—Aneurysm of the nonpatent ductus arteriosus in the newborn is a rare but potentially fatal condition Of reported ductal aneurysm up to 1969 diagnosis was made at autopsy only We report here to our knowledge for the first time on a two week old infant with correct preoperative angiographic diagnosis followed by a successful surgical cure

KEY WORDS Infantile ductal aneurysm

An aneurysm of the ductus arteriosus in the neonate in contrast to simple patency is very rare From the 60 cases reported in the literature (2 3 4 5 6 7) it seems that in no instance a correct preoperative diagnosis was followed by a successful surgical cure as was the case in our 2 weeks old infant

CASE REPORT

The 3980 g male newborn offspring of a 26-year-old I para showed radiological cardiomegaly together with a transient hypoglycemia (20 mg/100 ml) on the first day of life On the 5th day suddenly heart failure developed There was tachycardia (160/min) tachypnoe (100/min) and an enlarged liver 6 cm below the costal margin There were normal heart sounds and no murmurs The ECG showed an axis of +100° and no signs of hypertrophy The chest X ray (Fig 1) revealed a slight cardiomegaly and distinct round shadow in the upper left mediastinal region The baby was treated with digoxin and diuretics improved with this therapy and was catheterized 2 days later The saturations and pressures in both heart sides were normal On cineangiography from the left ventricle the only anomaly was a big aneurysm at the aortic end of the already closed ductus arteriosus (Fig)

The child continued to improve and on day 10 the signs of heart failure were virtually gone It was apparent that the congestive heart failure was not related to the ductal aneurysm but was possibly connected with hypoglycemia and cardiomegaly of infants of diabetic mothers Since rupture and thrombo-embolism of the ductal aneurysm were reported as dangerous complications (? 3 4) surgical removal was performed on the 14th day The size of this aneurysmal structure was 13×20 mm Its wall was thin and fragile and was in communication only with the descending aorta The pulmonary end was already a narrow string of connective tissue Histologically there was a fresh necrosis of the media of the ductus arteriosus The postoperative course was uneventful digoxin was discontinued a week later and a healthy child was discharged

COMMENT

Under normal circumstances the ductus arteriosus constricts shortly after birth and anatomical closure is completed within 5–7 days but may not occur up till 21 days Constriction and subsequent intimal fibrosis is observed initially at the pulmonary end then the closure extends also to the aortic site The wall of the ductus undergoes changes by a de

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Submitted Jan 24 1977
Accepted March 17 1977

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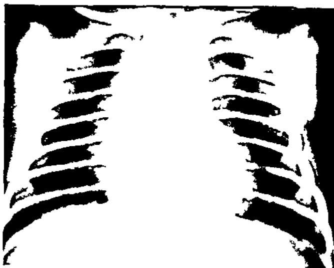


Fig 1 Angiography chest roentgenogram at 7 days of age demonstrates a slight cardiomegaly with a distinct round mass in the upper left mediastinum

crease in elastic fibres and by increase of fibrous connective tissue giving rise to the ligamentum arteriosum The aortic end of the ductus the ductal ampulla (ductal bump) may be seen roentgenologically and angiographically in many newborns for 1–4 days (1) If closure of the aortic end is delayed the ductus in effect becomes an aortic diverticulum In our case this formation might have been related to heart failure since hypoxia from low cardiac output could have interfered with physiological involution This diverticulum is under systemic pressure and along with the involution of the ductal tissue dilatation and aneurysm formation could be postulated Histologically the smooth muscle of the resected specimen in our case was necrotic whereas the other layers were normal Similar anatomical findings were reported in the literature (2 3 4) Although spontaneous uncomplicated involutions were described (6) rupture dissection thromboembolic complications and infections of the aneurysm were shown to account for a very high mortality (2 3 4) Thus up to 1969 the reported cases of ductal aneurysms in infancy were all diagnosed at autopsy The only infant where this malformation was recognized angiographically died shortly after the investigation (7) Two infants age 4 weeks and 12 days respectively were successfully operated but the indication for surgery was an intrathoracic mass of unknown origin (5)



Fig 2 Angiography from the left ventricle showing a big aneurysm at the aortic end of the ductus arteriosus

From our experience and from the reviewed literature (2 3 4 5 6 7) the following conclusions can be drawn Specific clinical symptoms are absent Roentgenographic examination is not diagnostic but a tumor like left sided mediastinal mass in the newborn is possibly an aneurysm of the ductus arteriosus Because no further involution can be expected but life threatening complications are the rule angiographical confirmation of the ductal aneurysm and prompt surgical removal are pertinent

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Submitted Jan 4 1977
Accepted March 17 1977

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Acta Pædiatr Scand 66 653–657 1977

CASE REPORT

THE LARSFN SYNDROME AND GLIAL PROLIFERATION IN THE BRAIN

PER HENRIKSSON STEN IVARSSON and GEORG THEANDER

From the Department of Paediatrics and the Department of Radiology (Paediatric Division) University of Lund Allmanna Sjukhuset Malmo Sweden

ABSTRACT Henriksson P Ivarsson S and Theander G (Department of Paediatrics and Department of Radiology (Paediatric Division) University of Lund Malmo General Hospital Malmo Sweden) The Larsen syndrome and glial proliferation in the brain Acta Paediatr Scand 66 653 1977.—A case of the Larsen syndrome is reported in which postmortem examination of the brain revealed glial proliferation resembling tuberous sclerosis The differential diagnosis of the syndrome and the possible significance of the lesions are discussed

KEY WORDS Larsen syndrome congenital knee dislocation glial proliferation tuberous sclerosis

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In 1972 Silverman (8) reported 5 personal cases of the Larsen syndrome and reviewed 41 which he had culled from the literature most of them had been published under various other diagnoses The same year Steel & Kohl (10) reported 3 siblings with the syndrome Several examples without specified sources were collected by Spranger et al (9) in their atlas of bone dysplasias Since then 5 cases have been reported by Payet (4) 3 by Robertson et al (7) and one by Swensson et al (11) The present paper adds one case in which unexpected lesions were found in the brain

CASE HISTORY

The patient was a newborn girl with no family history of bone disease or dysplasia Her mother aged 28 had 2 years previously had a spontaneous abortion preceded by two legal abortions The girl was born in the 36th week of gestation (vaginal delivery) after premature rupture of the membranes An excess of amniotic fluid was noted The placenta and umbilical cord were normal There was no history of exposure to drugs or ionising radiation during pregnancy

The girl weighed 2 100 g at birth She was moderately asphyctic and had biparietal cephalhematomas The skull was relatively large with a slightly prominent forehead low hairline a flat nasal bridge and eyes somewhat wide apart (Fig 1) Both knees were dislocated and markedly unstable There was a simian crease in the right hand and

Acta Pædiatr Scand 66 653–657 1977

CASE REPORT

THE LARSEN SYNDROME AND GLIAL PROLIFERATION IN THE BRAIN

PER HENRIKSSON STEN IVARSSON and GEORG THEANDER

From the Department of Paediatrics and the Department of Radiology (Paediatric Division) University of Lund Allmanna Sjukhuset Malmo Sweden

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Fig 1 Facial appearance at 5 days of age

deformities of the feet Auscultation revealed an abnormal second heart sound but no definite murmur The general condition of the baby rapidly deteriorated and she died after 13 days the main clinical symptoms having been those of cardiac failure

Radiologic findings

The chest and abdomen and the entire skeleton were radiologically examined on the 12th day of life The heart was considerably larger than normal and the pulmonary vasculature suggested a left to right shunt The skull was normal except for the parietal cephalhematomas and slight bossing of the frontal bone None of the tubular bones had ossification centres in their epiphyses and the pubic bones were unossified A thin layer of undermineralised bone was seen along all metaphyseal surfaces and at the periphery of the pelvic and tarsal bones There was a bilateral metatarsus deformity and both knees were unstable the position of the lower legs ranging between normal and anterior dislocation (Fig 2) Measurement of the tubular bones in the hands revealed a pattern profile consistent with the Larsen syndrome (Fig 3)

Laboratory findings

Examination of the blood and urine including urine chromatography revealed nothing abnormal and serologic tests for rubella and herpes virus toxoplasma listeria and cytomegalic virus were negative The caryotype was normal

Autopsy findings

The heart had a wide ventricular septal defect involving the mitral and tricuspid rings and valves The skull was normal except for the two cephalhematomas There was no sign of intracranial bleeding and the exterior of the brain appeared normal but dissection of the brain revealed three separate plaques of grayish discoloration resembling droplets of a wax candle Two of these plaques 4 and 7 mm in diameter respectively were situated in the left lateral ventricle and one sized 3 mm in the floor of the right lateral ventricle (Fig 4) All other internal organs were normal except for changes attributed to cardiac failure Dissection of the knees showed the cruciate ligaments to be elongated but no further abnormality was found in the joints

On histologic examination of the brain the plaques mentioned proved due to subependymal glial proliferation of a type described as phacomatous or hamartomatous

DISCUSSION

Conditions to be considered in the differential diagnosis of the Larsen syndrome include several malformations bone dysplasias and other inheritable diseases such as isolated congenital subluxation of the knee arthrogryposis, the Marfan and Ehler Danlos syndromes and the oto palato digital syndrome Most of these are as a rule easy to exclude According to Silverman (8) the combination of anterior dislocation of the knees and a characteristic facial appearance is sufficient evidence of the Larsen syndrome In the present case the diagnosis was further supported by the bilateral metatarsus varus deformity the malformation of the heart the pattern profile of the hands and the lack of ossification in the pubic bones

Deformities of the feet were as mentioned found in all 6 cases reported by Larsen et al (3) and have been a frequent finding also in the other cases of the syndrome Although the type of the anomaly has often not been specified both valgus and varus deformities are on record Similarly although cardiac malformation has been observed or suspected in several instances of the syndrome but not always specified this malformation has differed in nature from one case to another Ventricular septal defects have been observed in some of the previous cases

Pattern profile analysis of the tubular bones in the hand is a method of investigation elaborated by Poznanski et al (6) and found to be valuable in the discrimination between several kinds of bone dysplasias The reference curve given in Fig 3 represents the pooled measurements in several cases of the Larsen syndrome studied by Poznanski According to this author a similar pattern profile has been found only in the oto palato-digital syndrome (5)



Fig 2 1 days of age Absence of ossification in epiphyses and pubic bones Underm neralised zones in metaphyses and pelvic bones Metatarsus varus deformity Instability of knee joints (two lateral views of same knee)

The pubic bones seem to have appeared normal in most cases of the Larsen syndrome but they were unossified at birth in 2 of the 5 cases reported by Silverman (8) In one of these cases ossification of the pubic bones was found to begin between the ages of 5 1/2 months and 2 years but at 6 1/2 years it was still limited to the lateral parts of the



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Submitted Febr 8 1977
Accepted March 9 1977

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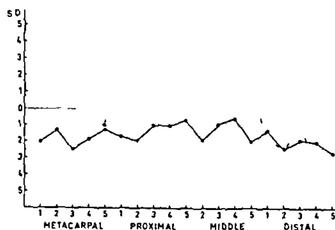


Fig 3 Pattern profile of tubular bone length in hand Continuous line curve obtained in the present case Broken line reference curve from pooled previous cases of the Larsen syndrome (5)

bones A similar delay in the ossification of these bones is a well known feature in cleido cranial dysplasia and in spondyloepiphyseal dysplasia, but then in combination with other osseous abnormalities not consistent with the Larsen syndrome

The absence of epiphyseal ossification centres in the present case might be explained by the low gestational age alone but this finding has been reported also in full term new born babies with the Larsen syndrome On the other hand the undermineralised zones seen in the metaphyses and small bones are generally considered an entirely non specific effect of disturbed metabolism In our case they may be attributed to the circulatory insufficiency after birth

Most of the findings made at autopsy also agree with previous reports The knees have been dissected in at least 3 previous cases of the Larsen syndrome (1 2, 3) In all of these the cruciate ligaments existed but appeared elongated and in one case the joint capsule was described as being abnormally wide On the other hand the lesions found in the brain were unexpected

The three plaques of glial proliferation situated subependymally in the lateral ventricles were macro and microscopically similar to the lesions seen in tuberous sclerosis but no such lesions were demonstrated elsewhere and no further characteristics of epiloia were present Neither tuberous sclerosis nor any other abnormality of the central nervous system is known to occur in the Larsen syndrome In one of the original cases described by Larsen et al (3) complete postmortem examination of the brain had been performed by a neuropathologist who discovered nothing remarkable This seems however to be the only previous case of the syndrome in which autopsy included a thorough examination of the brain It thus remains uncertain whether the glial proliferation was merely coincidental in the present case or should be considered one of the many facultative components of the Larsen syndrome



Fig 4 Photograph of left lateral ventricle of the brain Subependymal glial proliferations (arrows) in the lateral wall

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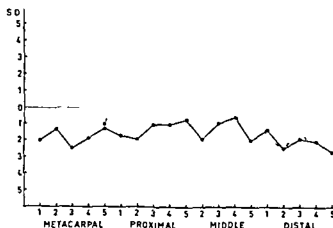


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Submitted Febr 8 1977
Accepted March 9 1977

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Acta Pædiatr Scand 66 659–662 1977

CASE REPORT

DELETION OF THE SHORT ARM OF CHROMOSOME NO 10

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ABSTRACT Berger R Larroche J C and Toubas P L (Centre de Recherches de Biologie due Developpement Foetal et Neonatal and Service de Medecine Neonatale Hopital Port Royal Paris France) Acta Paediatr Scand 66 659 1977 —A new case of deletion of the short arm of chromosome no 10 is reported The individualization of a new autosomal syndrome associated with a 10p- aberration is discussed

KEY WORDS Chromosomal aberrations chromosome no 10 deletion 10p

Banding techniques have made possible the description of new syndromes attributable to autosomal aberrations Among them a few cases of group C autosome deletion have been reported A new case of deletion of the short arm of chromosome no 10 is reported here

CASE REPORT

The propositus a male infant was born on April 76 1976 He was the first child of healthy unrelated parents The mother was 38 years old and the father 49 years old and both were in good health with no significant medical history The mother had a provoked abortion 14 years ago and a spontaneous miscarriage at 2 months gestation 4 years ago

The present pregnancy and delivery were normal Delivery occurred at 34–35 weeks of gestation the weight was 2700 g the length 49 cm and the head circumference 31 cm The Apgar score was 6 at 1 minute and 9 at 10 minutes Because of moderate respiratory distress the child was placed in an incubator

Numerous morphological abnormalities were noted (Fig 1) microphthalmia antimongoloid slant of the palpebral fissures hypertelorism epicanthal folds wide and flat nasal bridge everted nostrils prominent upper lip small and low set-ears with flattened helix prominent anthelix and adherent lobule micrognathia short neck widely spaced nipples dehiscent linea alba small penis left undescended testis club hands and club feet No dermatoglyphic abnormalities were seen



Fig 1 Front and side view of the propositus (post mortem)

Table 1 *Comparison of cases with deletion of the short arm of chromosome no 10*

	Elliot et al 1970	Francke et al 1975	Shokeir et al 1975	Present case
Sex	F	F	M	M
Parental age (m f)	25 34	25 25	22 22	38 49
Gestational age	35 w	Term	Term	34-35 w
Birth weight	1 780 g	3 300 g	2 530 g	2 200 g
Age		5 4/12 y		
Age at death	64 days		13 weeks	2 days
Growth retardation		+	+	
Antimongoloid slant	−	+	+	+
Epicanthal folds	−	+		+
Flat nasal bridge	+	+		+
Everted nostrils	+	−		+
High arched palate	+			
Cleft palate cleft lip			+	
Micrognathia	+		+	+
Small ears	−	+		+
Low set ears	−	+		+
Pre auricular pit or sinus	+		+	−
Prominent upper lip		+		+
Wide spaced nipples		+	+	+
Cardiac abnormalities	+	−	+	+
Urinary tract abnormalities	+	−	+	+
Genital abnormalities			+	+
Hernia	+	−	+	+
Hand and foot abnormalities	+	+		+
Olfactory bulbs and tracts abnormalities	+	+		+
Psychomotor retardation	+	+		
Karyotype	?10p-	del(10)p13	del(10)p13	del(10)p13
Parental karyotypes	normal	normal	normal	normal

been identified as 10p13 following Paris Conference (1971) nomenclature (Fig 2) The karyotype of both parents were normal

DISCUSSION

Four cases of constitutional deletion of the short arm of chromosome no 10 have been published One of them (1) is a monosomy for band 10p15 and a trisomy for bands 10q20 to 10q26 resulting from a maternal pericentric inversion Therefore the present case cannot be compared to the other cases Another infant (2) had been studied before banding techniques were available and no acurate identification of the deletion was possible In the two other cases (3 4 7) the break point was at band 10p13

The comparison of the phenotypes of 4 cases of 10p- (Table 1) shows that some abnormalities were found in several cases such as antimongoloid slant of the palpebral fissures flat nasal bridge, micrognathia widely spaced nipples cardiac and urinary tract abnormalities hernia hand and foot anomalies hypoplasia or absence of the olfactory bulbs and tracts Three out of four children died prematurely

Ten cases of trisomy for the short arm of chromosome no 10 are known (5 6 and 8 for review) Some of the abnormalities of the associated phenotype may be considered as antithetical (contretype) of some of those of the deletion phenotype such as prominent nasal bridge (versus flat) and large ears (versus small)

In spite of the small number of cases it appears that a new syndrome associated with a deletion of the short arm of chromosome no 10 may be recognized

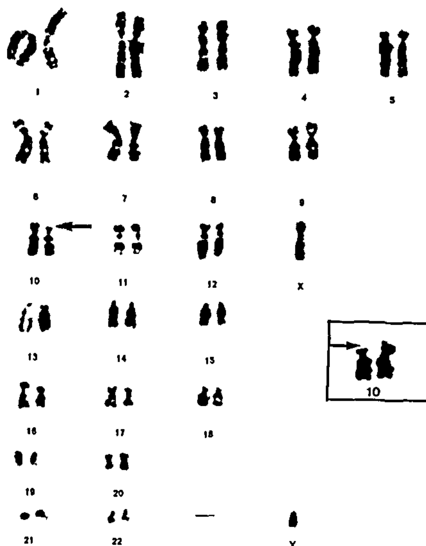


Fig 2 Karyotype (G bands) 46 XY del(10)(p13) In the frame magnification of the no 10 chromosomes

During the following hours the respiratory distress disappeared however the child remained cyanotic Complementary examination showed the existence of cardiac and urinary malformations Routine laboratory examinations were normal The child died on April 28 1976 at the age of 2 days

NECROPSY FINDINGS

The most salient features were as follows Visceral malformations dehiscent abdominal muscular wall (1 cm) truncus arteriosus hypoplasia and orificial atresia of the pulmonary artery absence of ductus arteriosus, intraventricular defect, abnormally large bronchial arteries abnormal histological aspects of the pulmonary arteries cystic dysplasia of the left kidney segmental dysplasia of the right kidney and common fibrous capsule of adrenal and kidneys Other pathological findings moderate amniotic fluid aspiration lobar hyaline membranes formation and slight degree of tubulopathy Brain abnormalities hypoplasia of the left olfactory bulb Growth morphological development was compatible with 36 weeks of gestation

LETTER TO THE EDITOR

Sir

Streptomycin and Isoniazid Metabolism in Malnourished Children

The paper by Akbani et al (*Acta Paediatr Scand* 66 237 1977) was of considerable interest as studies into drug metabolism in malnutrition are badly needed

However in the context of the abovementioned paper it should be pointed out that 2 problems arose which make data interpretation difficult Firstly the patients were only studied once namely when malnourished and not on recovery This would have allowed a comparison between admission and discharge status Secondly with regard to Streptomycin no data was provided with regard to protein binding and indeed serum albumin concentrations were not discussed

In the context of microbiological assays as were employed for the streptomycin assay it is the free (non protein bound) component of the drug which is measured Thus in hypoalbuminaemic patients assays should be performed against their own serum prior to antimicrobial therapy This does not appear to have been done in the present study and may well have affected the results This could have accounted for the relatively high peak levels seen at 30 minutes (mean 44 3 $\mu g/ml$)

To exemplify this statement studies from our laboratory using ^3H Dihydrostreptomycin sequesulphate with kwashiorkor serum (Albumin=2 4 g/100 ml) and normal serum (4 05 g/100 ml) in vitro have shown that streptomycin is 65% free in normal serum but 81% free in kwashiorkor serum (Fig 1) This could well have a significant effect on microbiological assays

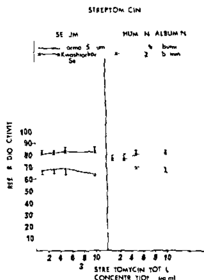


Fig 1 The binding of ^3H streptomycin to normal serum kwashiorkor serum and 4% and 2% human albumin The data represents the mean +1 S D

The present comments are not designed to detract from the value of the report in question but to draw attention to the technical problems associated with microbiological assays under such circumstances

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Submitted Nov 27 1976
Accepted Febr 1 1977

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Preparation of Manuscripts

Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts *Failure to follow these rules is a frequent cause of delay in the publication of articles and may result in rejection of otherwise acceptable manuscripts*

Manuscripts should be sent, in duplicate to the office of the Managing Editor Professor C G Bergstrand Department of Paediatrics Malmö Allmänna Sjukhus S-214 01 Malmo Sweden mailed flat and transmitted by first class mail They should be double spaced typewritten on one side of the paper with wide margins Authors should exercise particular care in the preparation notation and description of figures and tables Each table must be accompanied by a descriptive title typed above the table to which it refers A suitable legend must be provided for each figure Oversized original illustrations should be photographed and a print on glossy paper submitted A separate *title page* is necessary and should bear a) the title b) the initials and names of the authors c) the institution of origin Below the list of references the principal author should state his address to which the proofs are to be sent

A short *abstract* not exceeding 200 words must accompany each manuscript informing about problem, methods results and conclusions Unexplained abbreviations and references are not allowed The abstract must be typed on a separate sheet and styled as illustrated

ABSTRACT Köhler L and Holst K (Department of Paediatrics University Hospital Lund Sweden) Dental health of four year-old children Acta Pædiatr Scand 00

An unselected population of 1 567 four year-old children

KEY WORDS Pre school children caries gingivitis

A *summary* is usually not necessary but may be allowed if the author (or the Editor) finds it essential A summary should be typed immediately after discussion"

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1 Brecher G & Stohlman F Jr Humoral factors in erythropoietin *In* L M Tocantins & R Penn (eds) *Progress in hematology* Gruno & Stratton, New York 1959 p 110
2 Smith C A. *The physiology of the newborn infant* Thomas Springfield Ill 1967 3rd ed vol 2 p 120
3 Vedra, B & Ulrych, J. Anaerobiosis in normal and asphyxiated premature newborns 2 Four approaches *Acta Paediatr Scand* 49 129 1960

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ISSN 0001-656X

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ACTA
PÆDIATRICA
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Acta Pædiatr Scand 66 409–419 1977

TOTAL PARENTERAL NUTRITION IN INFANTS

Blood Levels of Glucose Lactate Pyruvate Free Fatty Acids Glycerol D-β Hydroxybutyrate Triglycerides Free Amino Acids and Insulin

B S LINDBLAD G SETTERGREN H FEYCHTING and B PERSSON

From the Department of Paediatrics Karolinska Institutet and the Unit of Paediatric Anaesthesiology
St Goran s Children s Hospital Stockholm Sweden

ABSTRACT Lindblad B S Settergren G Feychting H and Persson B (Department of Paediatrics Karolinska Institutet and the Unit of Paediatric Anaestesiology St Goran s Children s Hospital Stockholm Sweden) Total Parenteral Nutrition in Infants Acta Paediatr Scand 66 409 1977.—Two regimens (A and B) for TPN were designed to meet the requirements of newborn infants for calories amino acids fatty acids electrolytes trace elements and vitamins Both A and B included fat emulsion (Intralipid®) A contained fructose and glucose B glucose only A provided amino acids (Vamin®) in proportions similar to those of whole egg B similar to those of human milk All nutrients were given simultaneously into peripheral veins by constant infusion Nineteen patients (11 newborns 8 infants) were studied for 1–28 days Twelve infants recovered 7 died In none could TPN be regarded as the cause of death Treatment was complicated by sepsis in 5 infants During the course of treatment blood levels of substrates and insulin were measured before during and 30 min after discontinuation of TPN Highly raised concentrations of circulating substrates seen in 3 infants seemed to be related to a poor clinical condition rather than to the regimen used Infants in good condition tolerated TPN well Low levels of branch-chained amino acids and tendency to ketonemia when infusion was stopped suggested that minimal rather than optimal supply of energy and of amino acids in relation to energy was provided with both regimens Low insulin levels associated with elevated blood levels of substrates suggested that insulin administration to selected cases might be indicated Fructose (0 30 g/kg×hour) given with regimen A increased blood lactate concentrations Homocystinaemia appeared in 2 cases disappearance after excess vitamin B administration indicated increased B requirement

KEY WORDS Infants parenteral nutrition amino acids fatty acids glucose glycerol D-β-hydroxybutyrate lactate pyruvate triglycerides insulin

In 1968 Dudrick and co-workers demonstrated that parenteral nutrition based on amino acid and glucose without fat could provide adequate nutrients for growth and support positive nitrogen balance in sick infants (5) Later reports have supported the life saving potential of parenteral nutrition (also regimens including lipid emulsions) to selective groups of severely sick infants (2 8 18 25)

It is well recognized that total parenteral nutrition (TPN) may be associated with serious complications sepsis related to catheters or metabolic disorders related to the composition of the infusate (1 8 25) The incidence of such complications may be reduced by following recommendations given by experienced teams working with this type of therapy (2 6 8 25) However our present knowledge about the requirements of different nutrients—amino acids in particular—during infancy is limited One may well ask to what extent data on requirements of normal infants are applicable to se

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Table 1 *Clinical data of infants treated with TPN*

TPN regimen	Case no	Sex	Age	Duration (days)	Birth weight in parenthesis and weight at start of TPN (g)	Diagnosis	Outcome
A	1 (Mu)	M	2 days	13	(2 110) 1 980	SFD duodenal atresia and intrahepatic gall accumulation *hypoglycemia and transcient diabetes* staph sepsis	Died
	2 (Li)	M	3 days	2	(1 870)	LBW IRDS aerobacter sepsis	Discharged
	3 (K B)	M	4 days	7	(3 570) 3 080	Mb Down duodenal atresia	Discharged
	4 (Al)	M	14 days	6	(2 950) 2 550	Duodenal cyst	Discharged
	5 (Ol)	M	14 days	19	(2 810) 2 200	Intestinal angiomatosis	Discharged
	6 (Hj)	F	14 days	5	(2 360)	LBW hypoplastic left ventricle cardiac failure *E coli* sepsis	Died
	7 (Br)	M	2 5 months	5	(3 030) 3 260	Pyloric stenosis *reoperation*	Discharged
B	8 (Ka)	F	1 day	15	(2 610)	SFD ruptured omphalocele	Discharged
	9 (Hy)	M	3 days	14	(2 810) 2 250	Duodenal atresia gall fistula	Discharged
	10 (Be)	M	6 days	14	(3 100) 2 520	Ileal atresia	Discharged
	11 (Ze)	F	10 days	9	(3 130) 2 600	Oesophageal atresia duodenal stenosis	Discharged
	12 (Lu)	M	14 days	1	(2 370)	SFD neonatal asphyxia necrotic enterocolitis *Klebsiella* sepsis	Died
	13 (Ro)	M	2 months	12	(1 670) 2 830	LBW colon stenosis (3 reoperations)	Discharged
	14 (Th)	M	3 months	6	(3 530) 2 800	Colon stenosis (reop) postoperative hypovolaemia	Died
	15 (Si)	M	4 months	5	(2 980) 3 030	Mb Down endocardial cushion defect with pulmonary hypertension *Klebsiella sepsis*	Died
	16 (Fo)	M	5 months	11	(4 000) 4 260	Malabsorption intractable diarrhoea staph sepsis	Discharged
	17 (Na)	M	6 months	14	(2 800) 6 310	Malabsorption intractable diarrhoea	Discharged
	18 (Fr)	F	9 months	28	(2 690) 4 770	SFD malabsorption intractable diarrhoea recurrent *E coli* sepsis	Died
	19 (Bo)	F	10 months	4	(2 300) 4 500	SFD oesophageal atresia (reop) *urinary tract infection candida* sepsis	Died

TPN given at 3 occasions for 3 14 and 11 days with 8 and 45 day intervals

verely sick infants The tolerance to amino acids is largely unknown in liver heart kidney disease respiratory insufficiency trauma infection and undernutrition—all conditions prevailing among infants requiring TPN Low birth weight infants have low activities of certain amino acid degrading enzymes (17) and intolerance to amino acids could be suspected to occur during TPN An excessive or unbalanced supply of amino acids may also lead to accumulation of metabolites due to not yet fully developed kidney function We have recently demonstrated that the cerebral uptake of certain amino acids in infants is dependent on their arterial concentrations (21) It is known that elevation and imbalance of circulating amino acids may have harmful effects (9) particularly on the developing central nervous system (26) Thus nitrogen balance and growth are not sufficiently sensitive to be the only criteria to evaluate what could be considered as an optimal supply of amino acids and other nutrients to sick infants Due to the metabolic interrelationships between the different dietary components (calorie supply will increase amino acid tolerance *amino acid supply will increase the tolerance to water potassium and fat*) and evaluation of requirements

Table 2 *Composition of TPN*

Detailed composition of the different solutions used is given in Table 4

Regimen A The daily fluid requirement was given as
1/4 = Intralipid® 20%+ Lipovit for Infants 60 ml/liter Intralipid®
1/4 = Vamin® (amino acids Na K Ca Mg Cl and fructose)
2/4 = Fructose glucose (15+5%)+heparin 0 4 ml (2 000 IU) per liter sugar solution+60 ml PED electrolyte solution per liter sugar solution Further addition of 4 N NaCl and Postoperative Potassium Solution according to requirement with daily variation according to serum analysis

Regimen B The daily fluid requirement was given as
1/4 = Intralipid® 20%+ Lipovit for Infants 60 ml/liter Intralipid®
1/4 = Amino acid solution 4200 (amino acids in water)
2/4 = Glucose 10 or 20% + heparin 0 4 ml (2 000 IU) per liter glucose+"PED electrolyte solution 60 ml/liter glucose Further addition of 4 N NaCl and Postoperative Potassium Solution according to requirement with daily variation according to serum analysis

To both regimen Water Soluble Vitamin Mixture was given separately as i v injections corresponding to 10 ml/liter glucose solution given

Supply of the different nutrients with regimen A (including 14 ml POP solution/liter carbohydrate solution) when given as 100 ml/kg and 24 hours

Energy	CHO	55 kcal (57 cal%)=13 4 g CHO (+0 43 g glycerol)
	Fat	35 kcal (35 cal %)= 3 6 g fat (+0 2 g phosphatides)
	Amino acids	8 kcal (8 cal %)= 2 4 g amino acids=2 0 g "true protein = 2 1 g protein /100 kcal
	Total	96 kcal=307 kcal/g nitrogen

N	313 mg	S	1 6 mE
Na	1 7 mE	Heparin	100 IU
K	? mE	Vitamin A	0 1 mg
Mg²	0 3 mE	Vitamin D	2 5 µg
Ca	1 18 mE	Vitamin E	3 3 mg
P	0 86 mE	Vitamin K₁	0 05 mg
Cl	4 6 mE	Vitamin B₁	0 1? mg
Fe²	4 5 mE	Vitamin B₂	0 18 mg
Zn²	0 9 µE	Vitamin B	0 ? mg
Mn	1 5 µE	Nic amide	1 mg
Cu	0 45 µE	Panthothenic acid	1 mg
J	0 03 µE	Vitamin B₁₂	0 ? µg
F	? 25 µE	Folic acid	?0 µg
Al Mb Cr	0	Biotin	30 µg
Co in Vitamin B₁		Vitamin C	3 mg

Table 3 *Supply of amino acids in mg/kg per 24 hours with regimen A (Vamin®) and B (4200) when given as 100 ml/kg and 24 hours*

	A	B
Tryptophan	33	47
Lysine	130	187
Histidine	80	70
Arginine	110	137
Aspartic acid	137	137
Glutamic acid	300	237
Alanine	100	?10
Glycine	70	70
Proline	270	187
Serine	250	127
Threonine	100	1?0
Tyrosine	17	17
Phenylalanine	183	90
Isoleucine	130	103
Leucine	177	233
Valine	143	120
Cysteine/cystine	47	33
Methionine	63	43
Total	2 340	2 168

necessitates the simultaneous administration of all nutrients In order to evaluate some metabolic consequences of our regimens for TPN we have determined plasma amino acids and other circulating substrates and insulin both during the simultaneous administration of all nutrients and 30 min after discontinuation of the infusion The measurements were repeated at different occasions during the course of treatment

MATERIAL AND METHODS

Subjects

Nineteen infants were treated with TPN 11 newborns and 8 infants between 2 and 12 months of age The majority of the patients were critically ill with a variety of gastrointestinal disorders requiring surgical treatment Diagnosis age at the start of TPN duration of treatment and clinical outcome have been summarized in Table 1

Program of TPN

The program for TPN of infants were designed to cover known or recommended requirements (7 10) Two regimens were used A and B (Tables 2 and 3) which both provided similar amounts of lipid electrolytes trace elements vitamins and fluid but were slightly different with respect to the content of carbohydrate and amino acids Regimen A provided amino acids in proportions similar to those of whole egg (Vamin®) B similar to those of human milk ("4200") The relative composition of the two programs was 1/4 of fat solution 1/4 of amino acid solution and 2/4 of carbohydrate solution The detailed composition of the different solutions used including the addition of vitamins electrolytes and trace elements is given in Table 4 The amount of fluid given by continuous pump infusion

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Table 5 *Mean values ±S E M of circulating substrates in mmole/l determined before (n=6) during and 30 min after discontinuation of total parenteral nutrition (TPN)*

	Before TPN	During TPN	p^a	n^b	30 min after TPN
Glucose	7 6 ±1 9	5 0 ±0 5	<0 01	27	3 8 ±0 3
Lactate	1 98±0 32	? 18±0 21	<0 0?	25	2 03±0 23
Pyruvate	0 12±0 03	0 14±0 01	<0 01	13	0 12±0 01
FFA	0 89±0 71	0 73±0 11	n s	27	0 73±0 19
Glycerol	0 17±0 04	0 44±0 ?6	n s	??	0 58±0 4
D-β hydroxybutyrate	0 57±0 33	0 24±0 06	<0 05	27	0 31±0 11
Triglycerides	0 93±0 37	1 9 ±0 6	<0 01	?6	1 66±0 33
Insulin mU/l	5 9 ±0 1	7 4 ±? 5	n s	?0	3 8 ±1 2

p indicates statistical difference between paired values obtained during TPN and 30 min after TPN (paired *t* test)
n denotes number of paired observations

ording to the analytical results If during the course of treatment plasma became opalescent the amount of lipid was reduced by 50% or completely omitted

Blood sampling and analyses

Venous blood samples were drawn (always from a different vein than that used for the infusion) during treatment with TPN and 30 min after discontinuation of the infusion In a few instances venous blood samples were also collected before the institution of TPN Venous blood was drawn into a heparinized syringe and the sample was immediately centrifuged A portion of plasma was deproteinized with christalline sulphosalicylic acid The deproteinized samples were stored in −72°C until analysis Amino acids were analysed on a Biocal 00 (Munich) automatic aminoacid analyzer according to the method of Spackman et al (23) using a lithium buffer system (3) to enable separation of glutamine asparagine threonine and serine Plasma tryptophan levels are not reliably determined by this method due to the partial binding of tryptophan to albumin The reproducibility of the amino acid analyses varies between ±3 to ±6% with the exception of cystine determination (±10) the amino acid peak of which had a tendency to overlap with the buffer change through the extreme sensitivity to buffer pH variation This amino acid also has a tendency to disappear through handling and storage of the sample Glucose (glucose oxidase Glox® AB Kabi Stockholm Sweden) free fatty acids (14) glycerol (13) D-β-hydroxybutyrate (?0) triglycerides (15) and insulin (Phadebas® Pharmacia Uppsala Sweden) were determined on plasma Lactate and pyruvate were determined on whole blood (4)

RESULTS

Clinical course

Diagnosis and clinical course are summarized in Table 1 Septic complications occurred in 8 infants in 3 instances cases 15 18 and 19 sepsis was present already before the institution of TPN Seven infants died 6 during the treatment with TPN and one a considerable time after discontinuation of TPN In none of these infants could TPN be regarded as the cause of death as judged from clinical data and autopsy findings Cases No 1 and 6 died be

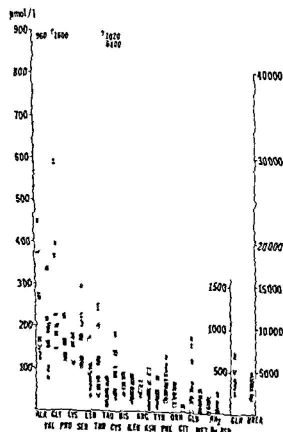


Fig 2 Individual values of plasma free amino acid levels during TPN Open circles represent values obtained with model A and closed with model B Mean±2 S D of a normal material are indicated (4) by horizontal lines and bars

Table 4 *The detailed compositions of the different solutions used*

1 Intralipid® (Vitrum) 20% soybean oil egg lecithine 27 mE $P^{1\ 8-}$/liter Tocopherol 200 mg/liter
2 Vamin® (Vitrum) 18 L amino acids 70 2 g=9 4 g N= 60 g ideal protein per liter The composition is according to the relationships between the different amino acids in whole egg Fructose 100 g/liter 650 kcal (410 from fructose) per liter Na^+ 50 mE K^+ 20 mE Ca^{2+} 5 mE Mg^2 3 mE (sulphate) Cl^- 55 mE per liter (At present available commercially with glucose instead of fructose)
3 Amino acid solution 4200 Chrystalline amino acids in water 65 g/liter

	Contents	
	Calculated	Test analysis (ion exchange chromatography) batch No 234202 (g/liter)
Tryptophan	1 4	1 33
Lysine	5 6	5 95
Histidine	2 1	2 14
Arginine	4 1	4 33
Aspartic acid	4 1	4 19
Glutamic acid	7 1	8 56
Alanine	6 3	6 24
Glycine	2 1	2 11
Proline	5 6	4 88
Serine	3 8	3 81
Threonine	3 6	3 63
Tyrosine	0 5	0 50
Phenylalanine	2 7	2 80
Isoleucine	3 1	3 31
Leucine	7 0	6 80
Valine	3 6	3 95
Cysteine/cystine	1 0	0 82
Methionine	1 3	1 43

The amino acid composition is according to the molar relationship found in human milk Due to technical reasons and in order to achieve neutrality the tyrosine aspartic acid and glutamic acid content is comparatively lower and the alanine and arginine content is comparatively higher than that in human milk

4 PED Electrolyte Solution Ca 3 mmole Mg 0 5 mmole Fe^{+++} 10 μmole Mn 5 μmole Zn 3 μmole Cu 1 5 μmole P 1 5 mmole F 15 μmole J 0 2 μmole Cl 6 3 mmole sorbitol 6 g sterile water to 20 ml
5 Lipovit for Infants Vitamin A 1 mg retinol (3333 IE) D 25 μg (1000 IE) K_1 0 5 mg soybean oil 1000 mg egg yolk phospholipids 120 mg glycerol 250 mg sterile water to 10 ml
6 Water Soluble Vitamin Mixture Thiamine 1 2 mg as mononitrate 1 236 mg riboflavine 1 8 mg as Na riboflavine phosphate 2 466 mg nicotinamide 10 mg pyridoxine 2 mg as pyridoxine chloride 2 431 folic acid 0 2 mg B_{12} 2 μg panthotenic acid 10 mg as sodium panthothenate 11 mg biotin 0 3 mg C 30 mg as sodium ascorbate 34 mg amino acetic acid (as body) 100 mg Lyophilized to be dissolved in 5 ml 10% glucose for intravenous infusion
7 POP® (ACO) Postoperative Potassium Solution K^+ 40 mE Ca^2 3 5 mE Mg^2 1 5 mE Cl 50 mE H_2O 21 ml

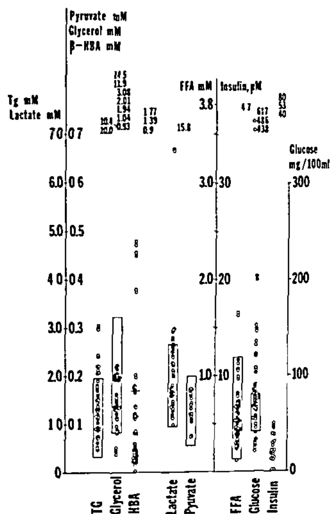


Fig 1 Individual values of plasma triglyceride glycerol hydroxybutyric acid lactate pyruvate free fatty acid glucose and insulin levels during TPN Open circles represent values obtained with model A and closed with model B Normal mean ±2 S D are indicated by open bars HBA and insulin levels do not show a normal distribution and the S D are therefore not indicated

into peripheral veins was 100 ml/kg×24 hours [1] The amount of calories electrolytes trace elements vitamins and essential amino acids supplied per 100 ml of infusate given is illustrated in Table 2 and 3 TPN which means the administration of hypertonic solutions was never started before corrections of water and electrolyte im balances had been made Treatment before TPN included infusion of carbohydrate solutions (10% glucose with added Na and K^+) When there was abundant loss of fluid from the gastrointestinal tract or when laboratory analyses indicated hemoconcentration the amount of carbohydrate solution of the infusate was increased Sodium and potassium were given as chloride in amounts corresponding to a concentration of 40 mE/liter of infusion Hemoglobin hematocrit base excess blood urea nitrogen (BUN) and plasma concentrations of sodium and potassium were determined daily When indicated the amount of sodium and potassium given was adjusted ac

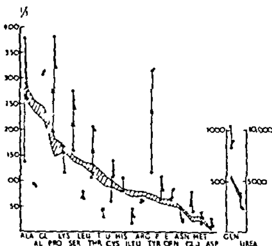


Fig 5 Plasma free amino acid levels before during and 30 minutes after cessation of TPN of a prematurely born human infant (case No) during the 4th day of life The striped area represents normal mean ±S E M (74)

concentrations moderately elevated levels of D-β hydroxybutyrate and low insulin values

Glucose and insulin values were significantly correlated ($p<0.001$) A correlation was also found between leucine and insulin ($p<0.001$) whereas no statistical relationship was found between isoleucine and insulin No statistical correlation was found between D-β hydroxybutyrate and glucose values or between alanine and D-β hydroxybutyrate values

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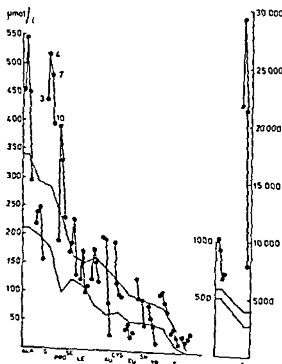


Fig 6 Plasma free amino acid levels before start of the TPN on the 3rd day of life and during the therapy on the 4th 7th and 10th day of life of case No 3 The horizontal lines represent mean ±S D of a normal material (4)

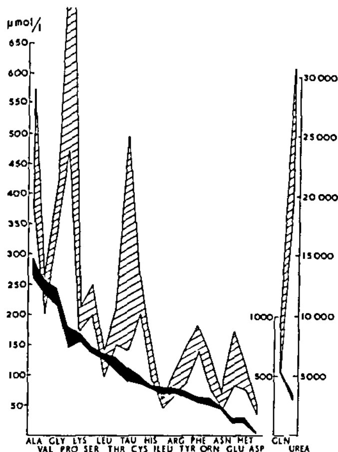


Fig 3 Plasma free amino acid levels during TPN in 3 cases (striped areas) where the therapy gave rise to a serious derangement of the stabile pattern seen under normal conditions (24) (filled area) Mean±S E M are indicated These results are included in Fig 2 and 4

cause of malformations incompatible with life case No 12 from necrotic enterocolitis complicated by sepsis case 15 from heart failure due to endocardial cushion defect and in cases 18 and 19 the primary cause of death was sepsis Thrombosis was seen in the only infant where a central vein was used for infusion (case No 18)

The clinical impression was that cases No 5 8 and 13 survived because of the treatment with TPN and that cases No 16 and 17 were greatly helped by this therapy

Metabolic findings

Indices of carbohydrate and lipid metabolism Individual values of glucose lactate pyruvate FFA glycerol D β hydroxybutyrate triglyceride and insulin during TPN with the two regimens are given in Fig 1 In several instances the values were above +2 S D of normal fasting values for infants (also given in Fig 1) Greatly elevated plasma concentrations of glucose FFA triglycerides and glycerol were often found together with elevations of one or more amino acids (in 7 out of 12 cases) Such elevations of circulating substrates were frequently associated with a poor clinical condition and seemed to be unrelated to the type of TPN regimen used Mean values of glucose lactate pyruvate FFA, glycerol D β hydroxybutyrate, triglycerides and insulin determined before during and 30 min after discontinuation of TPN are summarized in Table 5 During TPN the mean values fell within the normal range of fasting concentration with the exception of moderately elevated mean values of triglycerides and glycerol A comparison between values obtained during and 30 min after TPN using the paired *t* test showed a significant decline of glucose lactate pyruvate and triglycerides whereas D-β hydroxybutyrate values increased significantly A more pronounced drop of lactate was seen in patients receiving TPN regimen A where mean values decreased from 2 24 to 1 81 ($p<0\ 001$) Mean values 30 min after TPN was discontinued were within the normal fasting range with the exception of significantly elevated glycerol

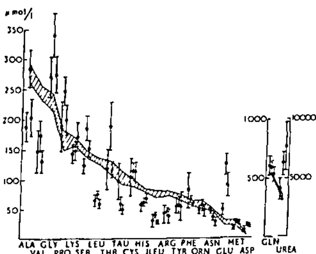


Fig 4 Mean±S E M of the plasma free amino acid levels of the whole material before during and 30 minutes after cessation of TPN The striped area represents normal mean ±S E M (24)

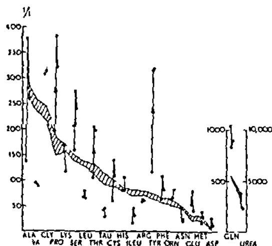


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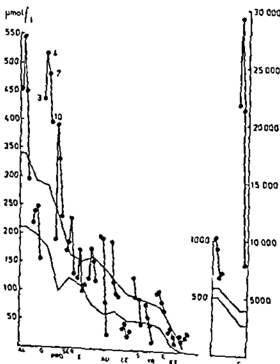


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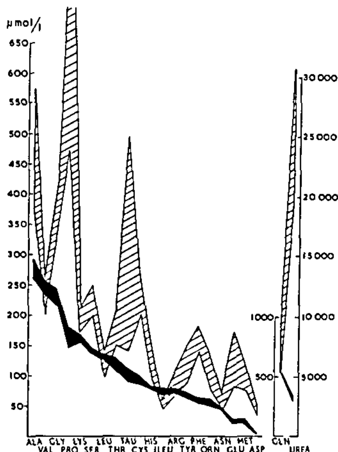


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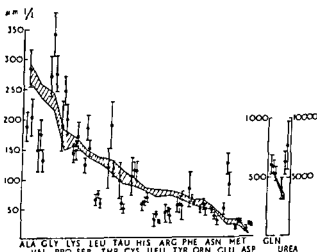


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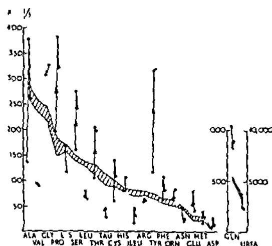


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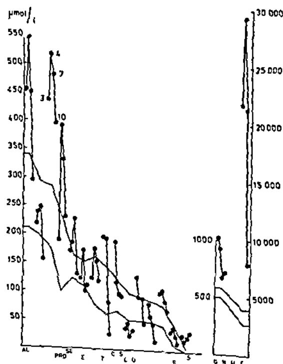


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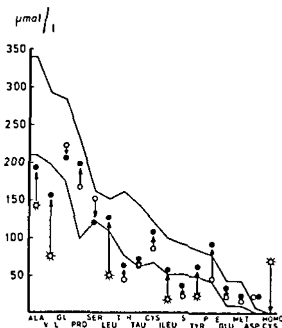


Fig 7 Plasma free amino acid levels of case 14 during TPN (open circles the low alanine branch chained and tyrosine levels characteristic of undernutrition and the homocystinaemia are indicated with stars) The filled circles represent the levels during TPN 2 days after the institution of vitamin B_6 (100 mg/day) Normal levels of infants mean ±S D (24) are indicated by the horisontal lines

ing buffer therapy was occasionally seen with both TPN regimens

Markedly elevated values of tyrosine proline serine and threonine were observed after 4 hours treatment with regimen A in case No 2 (Fig 5) Temporary elevations of some amino acids both before and after treatment for 24 hours are illustrated in Fig 6 (case No 3) As treatment continued the plasma concentrations of most amino acids decreased to the normal range and simultaneously the level of urea declined

Homocystine was detected in plasma in 2 infants One of these infants (case 14) also had low plasma concentrations of alanine valine leucine isoleucine and tyrosine during therapy with TPN (Fig 7) Following treatment with 100 mg/day of vitamin B_6 homocystine was no longer detectable in plasma and the plasma amino acid concentrations became normal within 24 hours (Fig 7)

Excessive taurinaemia of 6 500 µmole/l was seen in one icteric infant (case No 1) who was later found to have gall bladder agenesis and intrahepatic gall accumulation

DISCUSSION

The present material of very sick infants who required TPN was heterogenous with respect to age, diagnosis clinical condition, duration of treatment and number of determinations of circulating substrates and insulin TPN irrespective of the regimen used was well tolerated by the majority of infants studied However when the general condition of the infant was seriously affected with decreased peripheral circulation plasma levels of most amino acids, triglycerides glycerol FFA D-β hydroxybutyrate glucose and blood lactate were markedly elevated Therefore and because the clinical material was very heterogenous we have not performed a strict comparative evaluation of the two TPN regimens used

Before TPN was started all patients were given an electrolyte glucose solution intravenously This treatment provided only around 40 kcal/kg×24 hours^{-1} The finding of low plasma alanine concentrations elevated plasma levels of D β hydroxybutyrate FFA glycerol and glucose and low plasma insulin concentrations before TPN indicated a state of starvation During TPN the plasma concentrations of D-β hydroxybutyrate decreased by approximately 50% and the plasma alanine values became normal Already 30 min after discontinuation of TPN the plasma concentrations of D β hydroxybutyrate and alanine changed significantly D β hydroxybutyrate increased while alanine returned to a low level indicating enhanced hepatic ketogenesis and gluconeogenesis (16) None of the infants presented signs nor symptoms of hypoglycemia and the plasma insulin levels were generally low during TPN Hypoglucosemia i e a glucose value below 1 7 mmole/l was present in 3 infants (cases 1 5 19) Taken together these observations would suggest that minimal

rather than optimal amounts of carbohydrate were given

The finding of elevated blood lactate concentrations during treatment with regimen A which declined significantly 30 min after discontinuation of the infusion was most likely due to administration of fructose which was given at an approximate rate of 0 3 g/kg× hour^{-1} Our data and the fact that fructose administration could be extremely harmful in clinical situations such as hereditary fructose intolerance hepatic disease anoxic states and diabetic ketoacidosis (27) suggest that fructose should not be included in TPN The supply of lipid with regimens A and B was kept about 0 15 g/kg×hour^{-1} earlier demonstrated to be well tolerated by normal newborn infants (19) As long as the general condition of the infant was not too seriously affected this amount of lipid seemed to be well tolerated

Before TPN was started the plasma concentrations of leucine isoleucine and valine were low while the plasma glycine concentrations were high (Fig 4) This indicates protein deficiency (16) Low plasma concentrations of branch-chained amino acids have also been shown to be associated with impaired hepatic synthesis of albumin (12) However the plasma levels of leucine isoleucine valine and glycine did not become normal during TPN for an average period of 10 days suggesting either that the composition of amino acids in the infusate was inappropriate or more likely that a minimal rather than an optimal supply of essential nitrogen was given

Taurine asparagine and glutamine were not included in the infusate and the concentrations of these amino acids decreased in plasma during TPN The same was true for plasma concentration of cystine which was given in low amounts In general however the pattern of the plasma free amino acid levels did not mimic the composition of the infusate as has been suggested (24) but was rather characterized by a distortion similar to that seen in experimental and clinical undernutrition

Excessive supply of dibasic amino acids leads to metabolic acidosis (11) It is therefore possible that the slight metabolic acidosis seen in our patients was caused by a moderate overloading with dibasic amino acids A lowering of the relative amounts of these amino acids in the infusate should be considered—perhaps to a level which more closely mirrors the composition of human milk

In view of the raised demand for transport and metabolism of amino acids during TPN the need of an extra supply of vitamin B_6 as indicated by the findings in Fig 7 seems well founded A surplus of vitamin B_6 is recommended during TPN especially when given to severely undernourished infants

The present material included only one immature infant who required TPN during the neonatal period A poor tolerance to tyrosine administration—most likely due to low tyrosine amino transferase activity—is a particular problem in this situation (17) (Fig 5) The content of tyrosine was the same in both regimens and low in comparison to human milk

Elevated plasma concentrations of glycine and proline have been described in intrauterine growth retarded infants (16) Slightly elevated concentrations of plasma glycine and proline were seen in the present study both before and especially during TPN Since these amino acids can act as transmittor substances in the central nervous system (22) and theoretically could lead to neurological symptoms it is suggested that the content of glycine and proline in the infusate should be lowered

CONCLUSIONS AND RECOMMENDATIONS

Abdominal surgery in newborn babies and intractable diarrhea in infants are important indications for TPN during infancy In such situations TPN could be life saving In the case of abdominal surgery however TPN should be started early postoperatively TPN should not be given to desperate cases especially not if associated with decreased peripheral circulation Other contra indications include de

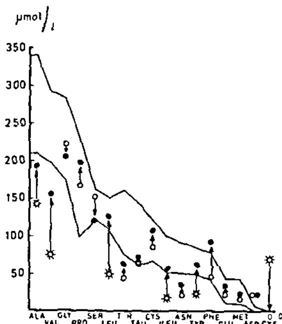


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hydration severely impaired liver and kidney function and coagulopathies It is our belief that TPN should only be given on very strict indications to newborn infants particularly if prematurely born The experience with TPN in this particular group of infants is limited and must still be regarded as being at an experimental stage hitherto unknown and undesirable metabolic and neurotoxic short and long term consequences should be further explored

The results of the present study suggested that fructose should be omitted from the infusate and that vitamin B_6 should be given in excess Furthermore the present results indicated that a minimal rather than an optimal supply of energy and of amino aicds in relation to energy was provided with the two regimens used It is therefore suggested that the total volume of the infusate could be raised above 100 ml/kg×hour⁻¹ during continuous monitoring of body weight This is in agreement with the higher recommendations of Børresen (2)

The marked increase in plasma concentrations of amino acids triglycerides glucose D β hydroxybutyrate seen in some cases could indicate a lack of insulin This was especially evident in one severely SFD born infant Insulin administration and monitoring of blood glucose should be considered in such cases rather than a decreased infusion rate

The amino acid composition of the infusion can probably be considerably improved to meet with the i v requirements Regimen B has in relation to human milk a rather low content of tyrosine and phenylalanine a composition that might be favourable for immature newborn infants If treatment with regimen B continues for a longer duration the content of phenylalanine should be increased in which case the amino acid solution of regimen A could serve as an alternative

ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance of Pharmacist B Ajaxon at the Galenic Developmental Laboratory of AB Vitrum who provided the electrolytical and vitamin solutions Professor A Wretlind of Karolinska Institutet gave us valuable advice during the planning of the intravenous program

This work was supported by grants from the Semper Fund for Nutritional Research from Karolinska Institutet from the Swedish Medical Research Council Projects Nos 19X 2583 19X 3787 and 19P-4106 and the Tielman Fund for Paediatric Research

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Submitted Oct 7 1976
Accepted Jan 1? 1977

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hydration severely impaired liver and kidney function and coagulopathies It is our belief that TPN should only be *given on very strict* indications to newborn infants particularly if prematurely born The experience with TPN in this particular group of infants is limited and must still be regarded as being at an experi mental stage hitherto unknown and undesirable metabolic and neurotoxic short and long term consequences should be further ex *plored*

The results of the present study suggested that fructose should be omitted from the infus ate and that vitamin B_6 should be given in ex cess Furthermore the present results indi cated that a minimal rather than an optimal supply of energy and of amino aicds in rela tion to energy was provided with the two regimens used It is therefore suggested that the total volume of the infusate could be raised above 100 ml/kg×hour^{-1} during continuous monitoring of body weight This is in agree ment with the higher recommendations of Bør resen (2)

The marked increase in plasma concentra tions of amino acids triglycerides glucose D β hydroxybutyrate seen in some cases could indicate a lack of insulin This was especially evident in one severely SFD born infant In sulin administration and monitoring of blood glucose should be considered in such cases rather than a decreased infusion rate

The amino acid composition of the infusion can probably be considerably improved to meet with the i v requirements Regimen B has in relation to human milk a rather low content of tyrosine and phenylalanine a com position that might be favourable for immature newborn infants If treatment with regimen B continues for a longer duration the content of phenylalanine should be increased in which case the amino acid solution of regimen A could serve as an alternative

ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance of Phar macist B Ajaxon at the Galenic Developmental Labora tory of AB Vitrum who provided the electrolytical and vitamin solutions Professor A Wretlind of Karolinska Institutet gave us valuable advice during the planning of the intravenous program

This work was supported by grants from the Semper Fund for Nutritional Research from Karolinska Institu tet from the Swedish Medical Research Council Pro jects Nos 19X 2583 19X 3787 and 19P-4106 and the Tielman Fund for Paediatric Research

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Submitted Oct 7 1976
Accepted Jan 12 1977

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Acta Pædiatr Scand 66 421–424 1977

ABNORMALITIES OF IMMUNOGLOBULINS IN INFANTS WITH CONGENITAL HEART DISEASE

A P COLE D PERRY and J R HOBBS

From the Westminster Hospital London England

ABSTRACT Cole A P Perry D and Hobbs J R (Westminster Hospital London England) Abnormalities of immunoglobulins in infants with congenital heart disease Acta Paediatr Scand 66 421 1977.—Twenty-eight infants had their serum immunoglobulins estimated (by radial immunodiffusion) in early infancy The IgG level was abnormal in twelve infants Elevated levels of IgA were found in ten and IgM in fourteen These variations mostly obvious in the first ten days of life were detected in the absence of clinical or immunological evidence of congenital rubella infection

KEY WORDS Immunoglobulins congenital malformations heart infancy

The connection between maternal rubella in the first trimester and congenital malformations has been known since 1941 (16) The diagnosis of a congenital infection with the rubella virus could be made on demonstrating a raised titre of rubella antibodies in the cord blood Epidemics of rubella may result in many affected infants with the characteristically raised IgM fraction persisting for months (8 15)

There has been speculation that other viruses could be teratogenic and the cytomegalovirus measles smallpox mumps vaccinia and varicella zoster poliomyelitis echo herpes and influenza and Coxsackie viruses have all been considered (2 10 11 12 14)

The ubiquitous nature of many maternal infections during pregnancy renders retrospective studies difficult to interpret and although twenty six viruses have proved foetopathic in animals (3 4) there is still no satisfactory animal model for the experimental production of congenital malformation of the heart

MATERIALS

Twenty-eight consecutive infants referred to three separate cardiac investigation units had their serum immunoglobulins estimated in a single laboratory Samples of blood were obtained during cardiac catheterisation or shortly afterwards In twelve cases these samples were obtained in the first two weeks of life and in none were the infants more than two months old

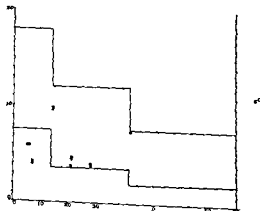


Fig 1 Serum levels of IgG against age in infants with congenital cardiac abnormalities The lines indicate the two S D limits of the log normal range for each block of age increases

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Fig 1 Serum levels of IgG against age in infants with congenital cardiac abnormalities The lines indicate the two S D limits of the log normal range for each block of age increases

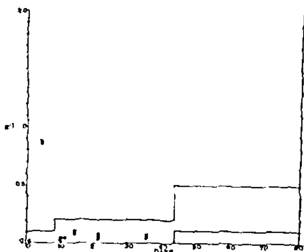


Fig 2 Serum levels of IgA against age in infants with congenital cardiac abnormalities The lines indicate the two S D limits of the log normal range for each block of age increases

The cardiac diagnoses (15 are listed in Table 1) were established by cardiac catheterisation or at necroscopy Some associated abnormalities were noted clinically or at necroscopy and in two infants multiple abnormalities conforming with the recognised Rubella syndrome occurred Virus cultures and rubella specific IgM estimations were performed and proved negative however

No infants were included in the study that had been diagnosed clinically bacteriologically or radiologically as suffering from an intercurrent infection No sera were obtained from infants after surgery

METHODS

The serum immunoglobulins were assayed by radial immunodiffusion (9) The method was calibrated using a pooled human serum which had been standardised against WHO Immunological Preparations (13) and pure human immunoglobulins The method has a between batch precision (coefficient of variation per cent) of IgG 5 2 IgA 8 4 IgM 8 5 Logarithmic normal ranges using the standard mg–International Unit contents of the WHO standards (13) and the three sections in Figs 1–3 represent ranges found for twenty five infants in the three age ranges

RESULTS

In the twenty eight sera examined the IgG level was subnormal for twelve (45%) ten of whom were under ten days old (see Fig 1) of these only two had premature birth by dates ten (40%) had raised IgA levels and of these five occurred within the first week of life (Fig 2), fourteen (50%) had raised IgM levels seven in the first two weeks of life (Fig 3) Since for the normal ranges used only 2 5% of infants should be expected either above or below the two S D limits shown, these findings are obviously highly significant Most of the diagnoses associated with raised immunoglobulin levels are shown in Table 1

DISCUSSION

The 45% incidence of subnormal IgG levels could not be explained by premature birth depriving the baby of the full maternal transfer as only two had been born prematurely nor by being small for dates due to obvious placental abnormalities It is possible that the cardiac defect itself impaired the placental circulation and thereby the transfer of IgG

The raised IgA and IgM levels especially the majority detected in the first few days after birth indicate immune stimulation within the infants (5 6)

The high incidence of raised IgA and IgM in infants with congenital heart disease provides non specific evidence in favour of intrauterine infection as a cause of abnormalities There was no maternal history pointing to any partic

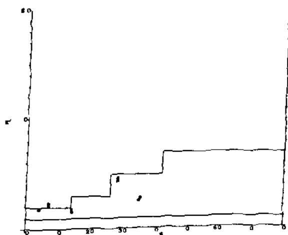


Fig 3 Serum levels of IgM against age in infants with congenital cardiac abnormalities The lines indicate the two S D limits of the log normal range for each block of age increases

Table I *Cardiac diagnoses in the infants with abnormal immunoglobulins*
P D A = patent ductus arteriosus V S D = ventricular septal defect

Cardiac diagnosis	Associated malformations	IgA raised	IgM raised	IgG raised	IgG reduced
P D A	Microcephaly	+	+	–	+
V S D P D A aortic valvular stenosis	Thymic hypoplasia	–	+	+	–
Pulm atresia P D A	–	–	+	–	–
Left heart syndrome	–	–	+	+	–
Extreme Fallot	Diaphragmatic eventration	–	+	+	–
Pulm atresia transposed aorta anomalous pulm venous drainage V S D	–	+	+	+	–
Anomalous pulm venous drainage	–	+	+	+	–
V S D pulm stenosis	–	–	+	+	–
Dextromension pulm atresia	–	+	–	–	–
Coarctation	Turner syndrome	+	+	–	–
Transposition of great vessels	–	–	+	–	–
Left heart syndrome	–	+	–	–	–
Coarctation P D A aortic valvular stenosis	Dislocated hips	–	–	+	–
V S D	–	–	–	+	–
V S D aortic stenosis	–	–	–	+	–

ular diagnosis but symptomless rubella and other viruses i e cytomegalovirus could be considered The high incidence of respiratory infection in infants with congenital heart dis ease could explain the findings but the con siderable proportion below the age of two weeks makes this rather unlikely On the other hand the finding of raised levels in individual patients with abnormalities very unlikely to be due to congenital infection such as coarctation in Turner s syndrome suggests that some other factors apply in some individuals

Finally it seems worth noting that when gamma globulin was given prophylactically to 3 366 mothers to be in order to protect them from rubella (1) it was found that there was less heart disease and other malformations than in the control population of mothers even when all those known to be affected by rubella were excluded This could suggest that gamma globulin had protected against other unknown viruses

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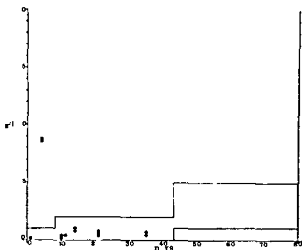


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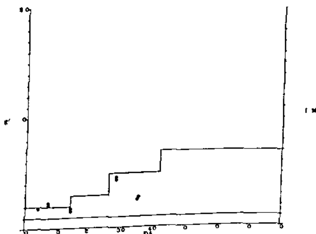


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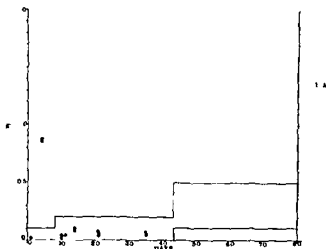


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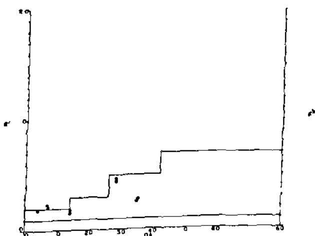


Fig 3 Serum levels of IgM against age in infants with congenital cardiac abnormalities The lines indicate the two S D limits of the log normal range for each block of age increases

Acta Pædiatr Scand 66 425–430 1977

ADAPTATION OF A SINGLE INJECTION CLEARANCE METHOD TO PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL FACTS

A Review of Data Obtained in Infancy and Childhood

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From the Division of Paediatric Nephrology University Children s Hospital Berne Switzerland

ABSTRACT Wassmer M Schafroth M Bretscher D Schneider A and Oetliker O (Division of Paediatric Nephrology University Children s Hospital Berne Switzerland) Adaptation of a single injection clearance method to physiological and pathophysiological facts Acta Paediatr Scand 66 425 1977.—A retrospective analysis of 395 Chromium ethylenediaminotetraacetate single injection clearances performed in infants and children is presented In 61% of infants and 30% of the children the clearance values were calculated on the basis of a plasma disappearance half time of the reference substance which was longer than the standard study i e on the basis of extrapolated data Plasma creatinine and urea levels were found to be appropriate indicators for predicting the plasma disappearance half time of the marker substance 14 additional patients were studied *prospectively with a dura*tion of the study predicted by means of the plasma creatinine and urea levels In these patients separate determinations of the clearances using either the data obtained during the standard time of procedure only or the data of the entire study clearly demonstrated that the clearances obtained by means of the standard procedure overestimated glomerular filtration rate The analysis of the data in infants show that the plasma urea level is a reasonably good indicator for predicting the time schedule of *the study whereas plasma creatinine* should not be used Additionally the retrospective data indicate that a prolongation of the study should be recommended in all infants This study demonstrates *the necessity and offers* means of adapting the time schedule of isotope single injection clearances to physiological and pathophysiological facts

KEY WORDS Glomerular filtration rate single injection clearances infants and children plasma creatinine plasma urea nitrogen

The procedure of single injection techniques to determine glomerular filtration rate is usually limited to a fixed time (2 4 19 21) *which is* determined by the mean plasma disappearance half time ($T_{\frac{1}{2}}$) of the reference substance Measuring plasma concentrations during 80–90 min allows in most instances the precise determination of $T_{\frac{1}{2}}$ (3 13) and thereby the calculation of glomerular filtration rate (GFR)

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Submitted Aug 20 1976
Accepted Dec 17 1976

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Acta Pædiatr Scand 66 425–430 1977

ADAPTATION OF A SINGLE INJECTION CLEARANCE METHOD TO PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL FACTS

A Review of Data Obtained in Infancy and Childhood

M WASSMER M SCHAFROTH[1] D BRETSCHER A SCHNEIDER and O OETLIKER[2]

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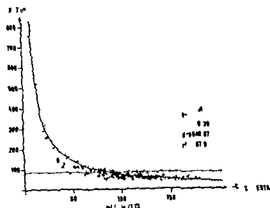


Fig 3 Plotting of plasma disappearance half time of Cr EDTA (Ti) against the clearance of [5] Cr EDTA (+=values obtained from 367 children ▲=values obtained from 28 infants) The calculated regression line is based on the values from children only The hatched area corresponds to the standard time during which measurements were obtained

Plasma creatinine was determined by the micromethod of Bartels (1) Plasma urea nitrogen was measured according to a modified urease/Berthelot method (17)

Analysis of data

In the 395 patients of the retrospective study the following relationships were examined

1 *Plasma creatinine concentration versus* Cr EDTA clearance

2 Plasma urea nitrogen concentration versus [5] Cr EDTA clearance

3 T_i versus [5] Cr EDTA clearance

4 T_i versus plasma creatinine concentration

5 T_i versus plasma urea nitrogen concentration

Regression lines were calculated for these relations according to the method of least squares using if necessary transformation of non linear curves $y=c+d/x$ to linear curves $y=c+d$ – (c_i=constant of regression line d_i–regression coefficient) Thereby it was possible to describe all examined relationships and to establish *for each* regression line the measure of determination (r^2) which describes the percentage of the total variability

The results of infants below 1 year of age were not subjected to a mathematical evaluation the *number of* patients being relatively small They were however individually plotted on the graphs

Prospective study

In the 14 renal insufficient patients in whom prospectively a prolonged measurement of the plasma disappearance half time of the test substance was performed the clearance values were obtained by two independent examiners in two different ways (*a*) using only the data of the first 85 min after the single injection of Cr EDTA (*b*) using the data found during the entire study

RESULTS

Frequency of plasma disappearance half time of ^{51}*Cr FDTA* ($T_{\frac{1}{2}}$) *being longer than 85 min*

The analysis of $T_{\frac{1}{2}}$ of 395 patients shows that in 61% of the infants and 30% of the children it was longer than 85 min Thus from the total number of observations one third of glomerular filtration rates were calculated on the basis of extrapolated data

Plasma creatinine concentration versus ^{51}*Cr EDTA clearance*

Fig 1 represents the relation between plasma creatinine concentration and the ^{51}Cr EDTA clearance An inverse relationship is observed In this graph it is quite obvious that almost all values of infants (triangles) are below the regression curve indicating that at comparable rates of glomerular filtration the plasma creatinine concentration in infants is usually lower than in children

Plasma urea nitrogen concentration versus ^{51}*Cr EDTA clearance*

Fig 2 shows the inverse relationship between plasma urea nitrogen concentration and ^{51}Cr

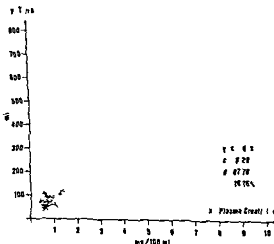


Fig 4 Plotting of plasma disappearance half time of ^{51}Cr EDTA ($T_{\frac{1}{2}}$) against plasma creatinine concentrations (+=values obtained from 157 children ▲=values obtained from 10 infants) Regression line calculated from the values of children only

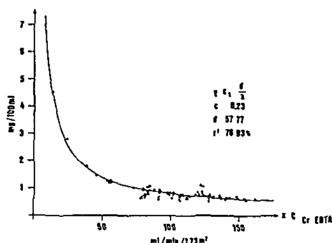


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late with GFR (7 9 10 11 20) it seemed likely that these parameters also correlate with the plasma disappearance half time of the reference substance Thus it might be possible to predict individually the time schedule of a single injection clearance study on the basis of plasma creatinine and/or urea concentrations

395 individual determinations of GFR by means of a single injection of ^{51}Chromium ethylenediaminotetraacetate (^{51}Cr EDTA) were analyzed mostly as a retrospective study A small number of renal insufficient patients was prospectively studied Practical conclusions may be drawn from this study improving the quality of determination of glomerular filtration rate by single injection techniques

MATERIAL AND METHODS

Patients

During three consecutive years 395 patients aged 1½ months–16 years had determinations of glomerular filtration rate by means of single injection of ^{51}Cr EDTA These patients either had mild to severe renal insufficiency or renal insufficiency was suspected or renal function needed to be documented for other reasons

Table 1 shows the 395 patients divided into different groups according to whether or not plasma values of creatinine and/or urea nitrogen were determined immediately prior to the study The results of 28 infants below 1 year of age and 367 children aged 1–16 years were retrospectively studied An additional 14 studies were performed prospectively in 14 patients with renal insufficiency in whom after the standard procedure the time of observation was prolonged up to 8 hours (Table 1)

Table 1 *Selection of patients for analysis of the correlation between plasma creatinine and/or urea nitrogen levels and T½ of ^{51}Cr EDTA*

Tabulation of 395 patients in whom glomerular filtration rate was determined The patients are grouped according to age and according to whether or not determination of plasma concentrations of creatinine and/or urea nitrogen were available at the time of the single injection clearance

Plasma concentration measured		*Number of patients*		
Creatinine	Urea nitrogen	<1 y	1–16 y	<1y –16y
+	+	7	136	143
+	–	3	11	14
–	+	5	43	48
–	–	13	177	190
Total number of patients		28	367	395

Methods

A standardized technique for determination of GFR by single injection of ^{51}Cr EDTA was used as described by Donath (4 15 16) Its comparability with the classical clearance technique was tested in our laboratory (21) All patients were studied in a recumbant position Comparable handling was warranted by the fact that they were all examined in a metabolic ward run by specially trained nursing staff For each study 8 blood samples were obtained during the 85 min after injection of the reference substance in 395 patients

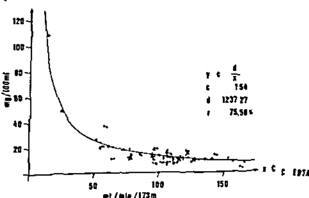


Fig 2 Plotting of plasma urea nitrogen concentrations against $C^{51}Cr$ EDTA (+=values from 191 children ▲= values from 12 infants) The calculated regression line is based on the values from children only

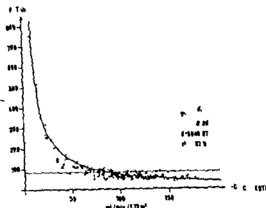


Fig 3 Plotting of plasma disappearance half time of ^{51}Cr EDTA (T½) against the clearance of ^{51}Cr EDTA (+=values obtained from 367 children ▲=values obtained from 8 infants) The calculated regression line is based on the values from children only The hatched area corresponds to the standard time during which measurements were obtained

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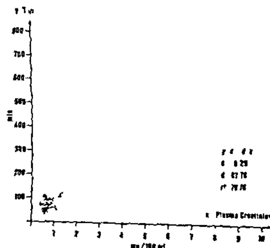
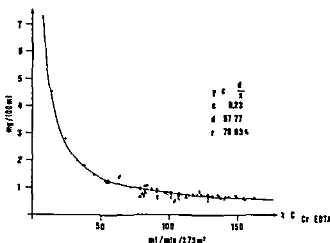


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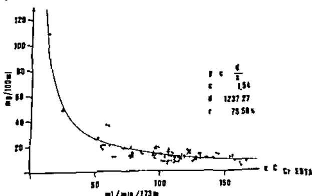
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Fig 2 Plotting of plasma urea nitrogen concentrations against C^51Cr EDTA (+ = values from 191 children ▲ = values from 12 infants) The calculated regression line is based on the values from children only

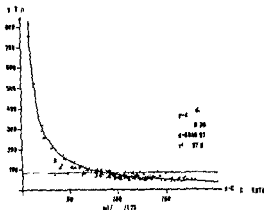


Fig 3 Plotting of plasma disappearance half time of ⁵¹Cr EDTA (T½) against the clearance of ⁵¹Cr EDTA (+ = values obtained from 367 children ▲ = values obtained from 78 infants) The calculated regression line is based on the values from children only The hatched area corresponds to the standard time during which measurements were obtained

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Analysis of data

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2 Plasma urea nitrogen concentration versus ⁵¹Cr EDTA clearance

3 $T_{½}$ versus ⁵¹Cr EDTA clearance

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RESULTS

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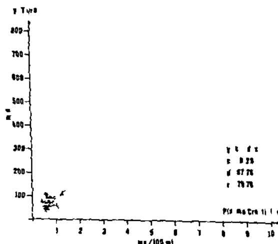


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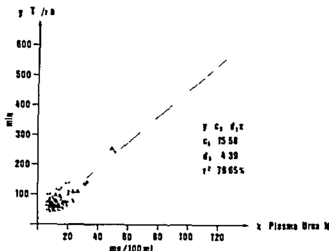


Fig 5 Plotting of plasma disappearance half time of ^{51}Cr EDTA ($T_{\frac{1}{2}}$) against plasma urea nitrogen concentration (+=values obtained from 191 children ▲=values obtained from 12 infants) *Regression line calculated from* the values of children only

EDTA clearance The values of infants (triangles) do not seem to differ from the values of children

T½ versus ^{51}Cr-EDTA clearance

Fig 3 shows the relation between the observed $T_{\frac{1}{2}}$ and the calculated ^{51}Cr EDTA clearance It can be seen that $T_{\frac{1}{2}}$ is inversely proportional to the clearance of ^{51}Cr-EDTA The values of infants represented by the triangles are clustered around this regression curve if GFR is near to normal adult values However when GFR is smaller than 60–50 ml/min per 1 73 m² the values are all situated towards the left side of the curve indicating that in the presence of the low GFR in this age group $T_{\frac{1}{2}}$ is shorter in infants than in children

T½ versus plasma creatinine and plasma urea nitrogen concentrations

The relationship between $T_{\frac{1}{2}}$ and plasma creatinine and urea nitrogen concentrations respectively are shown in Fig 4 and Fig 5 The relationship in infants between $T_{\frac{1}{2}}$ and plasma creatinine concentration (triangles Fig 4) seems to differ from the data obtained from children indicating rather longer $T_{\frac{1}{2}}$ despite low plasma creatinine concentration Such a difference between infants and children in the relationship of $T_{\frac{1}{2}}$ and plasma urea nitrogen concentration is not observed (triangles Fig 5)

Standard single injection clearance versus adapted technique in children with reduced GFR

In Fig 6 the correlation is shown between clearance values of children with reduced GFR calculated on the basis of $T_{\frac{1}{2}}$ as read from a decay curve described by data obtained during the 85 minutes following single injection (standard technique) and $T_{\frac{1}{2}}$ as read from a decay curve described by all data obtained in the same but prolonged study (adapted technique) (Fig 6) All points lie above the expected line of identity indicating persistantly higher clearance values when the standard technique was used to estimate GFR

DISCUSSION

The retrospective analysis of 395 patients in whom ^{51}Cr-EDTA single injection clearances were performed shows one of the major pitfalls of such a standardized procedure Using the standard technique the plasma disappearance curve of the injected test substance is

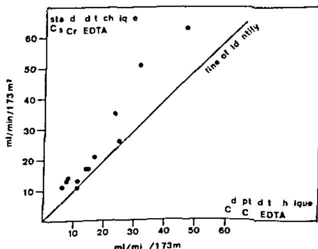


Fig 6 Correlation between clearance values calculated from the disappearance curves of ^{51}Cr EDTA as they presented on the basis of values obtained during the 85 min after injection of ^{51}Cr EDTA (standard technique) and on the basis of the entire prolonged study (adapted technique) *Each point thus represents a pair of data obtained* from one study

only measured up to 85 min This arbitrary time limit is based on the assumption that the plasma disappearance half time of the test substance is found within these 85 min However this assumption is often wrong and plasma disappearance half time exceeding 85 min have to be read by extrapolation This was necessary in 30% of children i e in the cases of moderate to severe renal insufficiency and in 61% of the infants i e in all infants including those with appropriate renal function Extrapolation automatically multiplies the minimal error of a decay curve constructed from the measured values In addition during the first 85 min of a study a pseudo-equilibration of the test substance might be observed yielding shorter than appropriate plasma disappearance half time and consecutively higher than actual GFR s This is most likely the explanation for the findings in an earlier study from this unit (21) where the single injection technique was shown to overestimate true GFR (clearance of inuline) when GFR was below 70 ml/min per 1 73 m² The 14 prospectively studied patients with GFR s below 50% of normal reported in the present paper confirm that equilibration of the test substance is incomplete after 85 min since all but one study led to higher GFR values when only the measurements during the standard 85 min were used Clearances calculated from extrapolated decay curves seem to be unreliable and it is concluded that an adaptation of the timing of ^{51}Cr EDTA clearance studies is necessary in order to obtain results more closely representing true GFR

In the present work possible indicators were examined which would allow to predict the approximate plasma disappearance half time before the clearance study An inverse proportionality between plasma creatinine and urea nitrogen concentrations respectively and glomerular filtration rate was demonstrated This relationship found in the paediatric age group is very similar to the data published by Mertz et al for adults (9 10) Similarly an inverse relationship was found between plasma disappearance half time and clearance of ^{51}Cr EDTA Therefore it was expected to find a linear relationship between $T\frac{1}{2}$ and plasma creatinine and $T\frac{1}{2}$ and plasma urea nitrogen concentration respectively This relationship was found indeed and it allows to predict with useful precision the plasma disappearance half time from plasma creatinine and/or plasma urea nitrogen concentrations It is suggested therefore to measure plasma creatinine or urea nitrogen concentration or both prior to the single injection clearance study The time schedule for an individual study can then be read from a graph as presented in Figs 4 and 5 According to the present data either indicator can be used with about the same adequacy Adapting the time schedule of the study to the predicted plasma disappearance half time makes extrapolation unnecessary or it prevents at least multiplying mistakes in constructing the plasma disappearance curve Thus it eliminates the most important pitfalls of performing single injection clearances

Infants exhibited lower plasma creatinine concentrations than children when compared with corresponding ^{51}Cr EDTA clearances The values were mostly below the regression line obtained from children reflecting the physiological finding that plasma creatinine levels are lower in infants than in older children (5 8) and the low plasma creatinine concentrations of infants are normally associated with a relatively low glomerular filtration rate (6 17 22) This is most likely due to the lower muscle mass of infants (14 23) Such a difference between children and infants was not observed for the relationship between plasma urea nitrogen concentrations and GFR Since the plasma urea level is very much dependent of the diet (18) nutritional factors might explain this observation Thus in infants plasma urea nitrogen concentrations might be used in a similar way as in children to predict the time schedule of a single injection clearance study Plasma creatinine concentrations however do not allow to predict the time schedule in infants

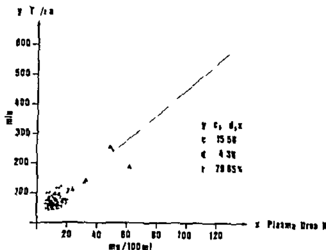


Fig 5 Plotting of plasma disappearance half time of [51]Cr EDTA (*T½*) against plasma urea nitrogen concentration (+=values obtained from 191 children ▲=values obtained from 12 infants) Regression line calculated from the values of children only

EDTA clearance The values of infants (triangles) do not seem to differ from the values of children

T½ versus [51]Cr EDTA clearance

Fig 3 shows the relation between the observed *T½* and the calculated [51]Cr-EDTA clearance It can be seen that *T½* is inversely proportional to the clearance of [51]Cr EDTA The values of infants represented by the triangles are clustered around this regression curve if GFR is near to normal adult values However when GFR is smaller than 60–50 ml/min per 1 73 m² the values are all situated towards the left side of the curve indicating that in the presence of the low GFR in this age group *T½* is shorter in infants than in children

T½ versus plasma creatinine and plasma urea nitrogen concentrations

The relationship between *T½* and plasma creatinine and urea nitrogen concentrations respectively are shown in Fig 4 and Fig 5 The relationship in infants between *T½* and plasma creatinine concentration (triangles Fig 4) seems to differ from the data obtained from children indicating rather longer *T½* despite low plasma creatinine concentration Such a difference between infants and children in the relationship of *T½* and plasma urea nitrogen concentration is not observed (triangles Fig 5)

Standard single injection clearance versus adapted technique in children with reduced GFR

In Fig 6 the correlation is shown between clearance values of children with reduced GFR calculated on the basis of *T½* as read from a decay curve described by data obtained during the 85 minutes following single injection (standard technique), and *T½* as read from a decay curve described by all data obtained in the same but prolonged study (adapted technique) (Fig 6) All points lie above the expected line of identity, indicating persistantly higher clearance values when the standard technique was used to estimate GFR

DISCUSSION

The retrospective analysis of 395 patients in whom [51]Cr EDTA single injection clearances were performed shows one of the major pitfalls of such a standardized procedure Using the standard technique the plasma disappearance curve of the injected test substance is

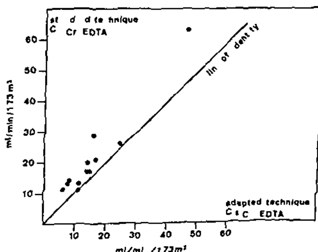


Fig 6 Correlation between clearance values calculated from the disappearance curves of [51]Cr EDTA as they presented on the basis of values obtained during the 85 min after injection of [51]Cr EDTA (standard technique) and on the basis of the entire prolonged study (adapted technique) Each point thus represents a pair of data obtained from one study

only measured up to 85 min This arbitrary time limit is based on the assumption that the plasma disappearance half time of the test substance is found within these 85 min However this assumption is often wrong and plasma disappearance half time exceeding 85 min have to be read by extrapolation This was necessary in 30% of children i e in the cases of moderate to severe renal insufficiency and in 61% of the infants i e in all infants including those with appropriate renal function Extrapolation automatically multiplies the minimal error of a decay curve constructed from the measured values In addition during the first 85 min of a study a pseudo equilibration of the test substance might be observed yielding shorter than appropriate plasma disappearance half time and consecutively higher than actual GFR s This is most likely the explanation for the findings in an earlier study from this unit (21) where the single injection technique was shown to overestimate true GFR (clearance of inuline) when GFR was below 70 ml/min per 1 73 m² The 14 prospectively studied patients with GFR s below 50% of normal reported in the present paper confirm that equilibration of the test substance is incomplete after 85 min since all but one study led to higher GFR values when only the measurements during the standard 85 min were used Clearances calculated from extrapolated decay curves seem to be unreliable and it is concluded that an adaptation of the timing of ⁵¹Cr EDTA clearance studies is necessary in order to obtain results more closely representing true GFR

In the present work possible indicators were examined which would allow to predict the approximate plasma disappearance half time before the clearance study An inverse proportionality between plasma creatinine and urea nitrogen concentrations respectively and glomerular filtration rate was demonstrated This relationship found in the paediatric age group is very similar to the data published by Mertz et al for adults (9 10) Similarly an inverse relationship was found between plasma disappearance half time and clearance of ⁵¹Cr EDTA Therefore it was expected to find a linear relationship between $T_{\frac{1}{2}}$ and plasma creatinine and $T_{\frac{1}{2}}$ and plasma urea nitrogen concentration respectively This relationship was found indeed and it allows to predict with useful precision the plasma disappearance half time from plasma creatinine and/or plasma urea nitrogen concentrations It is suggested therefore to measure plasma creatinine or urea nitrogen concentration or both prior to the single injection clearance study The time schedule for an individual study can then be read from a graph as presented in Figs 4 and 5 According to the present data either indicator can be used with about the same adequacy Adapting the time schedule of the study to the predicted plasma disappearance half time makes extrapolation unnecessary or it prevents at least multiplying mistakes in constructing the plasma disappearance curve Thus it eliminates the most important pitfalls of performing single injection clearances

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The plasma disappearance half time in infants was shown to be frequently longer than 85 min even with normal GFR s for age This observation implies that in all infants it is advisable to extend the time of study up to 120 min Predicting the time schedule by means of the demonstrated indicators and adapting the time schedule of the study to the frequently observed long plasma disappearance half time in infants improves the quality of measuring GFR by means of a single injection technique in children with any degree of renal insufficiency as well as in infants The presented adaptation of the single injection clearance study is based on physiological and pathophysiological facts which have to be accounted for

ACKNOWLEDGEMENT

The authors wish to thank Miss Brigitte Howald and Miss Christa Luthy for their most valid technical assistance

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Submitted May 6 1976
Accepted Jan 10 1977

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Acta Pædiatr Scand 66 431–437 1977

SOCIO PSYCHOLOGICAL FACTORS AND METABOLIC CONTROL IN JUVENILE DIABETES

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ABSTRACT Ludvigsson J (Department of Paediatrics Linkoping University Linkoping Sweden) Socio psychological factors and metabolic control in juvenile diabetes Acta Paediatr Scand 66 431 1977.—The influence of exogenous and environmental factors on metabolic control was studied in 58 insulin treated juvenile diabetics 6–17 years of age Duration of diabetes varied between 3–14 years and age at onset of diabetes between 1–13 years The social situation as well as knowledge about and attitudes towards diabetes among the patients and their parents were estimated by interviews questionnaires and special tests The quality of the diet exercise and insulin treatment was assessed An index of diabetic control was calculated on the basis of the patients daily urinalysis made at home Multiple regression analysis and a special statistical instrumental variable technique were used in an effort to analyse the correlations between all variables The social situation of the diabetic children was comparable with that of other Swedish children but many parents felt the economic burden of the diabetic treatment as a problem Knowledge tests showed that 25% of the parents and 62% of the patients above 12 years had unsatisfactory knowledge about diabetes However 93% of the patients seemed to have predominantly positive attitudes towards the treatment Severe psychological problems had occurred in 7 cases Food habits were appropriate among 71% of the patients and 26% had very regular exercise customs Physical exercise seemed to be the most important of the exogenous factors for the diabetic control ($p<0.001$) Among teenagers knowledge was positively correlated to positive attitudes which in turn were positively correlated to physical exercise Instrumental variable technique gave further indications of a positive influence of knowledge on control and the correlation between diabetic control on one hand and knowledge combined with positive attitudes on the other was significantly positive The results emphasize the importance of assisting young diabetic patients and their families in their socio-psychological adaptation to the strains of diabetic therapy

KEY WORDS Juvenile diabetes metabolic control treatment social situation knowledge attitudes physical exercise C peptide insulin antibodies

Current clinical and experimental data demonstrate that optimal regulation of blood glucose should be the goal in the treatment of diabetes particularly in order to prevent or minimize the development of microvascular lesions (4 7 27) It is well appreciated however that in some patients with juvenile onset diabetes such control of hyperglycemia is very difficult to achieve Many pathophysiological and environmental mechanisms obviously contribute to the maintenance of an adequate degree of diabetic control (21 28) Among endogenous factors of importance we have in a previous study demonstrated that persisting beta cell function approximately represented by measurable amounts of serum C peptide was positively correlated to a better diabetic control while a high level of insulin antibodies was negatively correlated to good control (25) The aim of the present study was to analyse in what way exogenous and environmental factors such as the quality of treatment

The plasma dısappearance half tıme ın ın fants was shown to be frequently longer than 85 mın even wıth normal GFR s for age Thıs observatıon ımplıes that ın all ınfants ıt ıs ad vıseable to extend the tıme of study up to 120 mın Predıctıng the tıme schedule by means of the demonstrated ındıcators and adaptıng the tıme schedule of the study to the frequently observed long plasma dısappearance half tıme ın ınfants ımproves the qualıty of measurıng GFR by means of a sıngle ınjectıon technıque ın chıldren wıth any degree of renal ınsuffı cıency as well as ın ınfants The presented adaptatıon of the sıngle ınjectıon clearance study ıs based on physıologıcal and patho physıologıcal facts whıch have to be ac counted for

ACKNOWLEDGEMENT

The authors wısh to thank Mıss Brıgıtte Howald and Mıss Chrısta Luthy for theır most valıd technıcal assıstance

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Submıtted May 6 1976
Accepted Jan 10 1977

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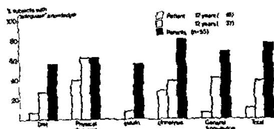


Fig 1 Knowledge about diabetes among 55 juvenile diabetics and their parents

tients >12 years old severe psychological problems diet *physical exercise* insulin dosage (IU/kg body weight) insulin frequency (1 or 2 doses a day) square root of fasting serum C peptide insulin binding capacity of IgG and index of *diabetic control during 1975*

In an effort to analyse what is cause and effect in the correlations between the variables in this cross-sectional study a special statistical technique using instrumental variables has been employed This method is discussed in detail elsewhere (22) As insulin injections and visits to the diabetic clinic were carried out by all patients irrespective of the patients attitudes these two items of the attitude tests were excluded from the statistical evaluation

RESULTS

Social situation The families belonged to social group I in 6 cases (10 3%) social group II in 18 cases (31 0%) and social group III in 34 cases (58 6%) The homes were broken in 5 cases Two patients lived in foster homes All fathers worked outside home but so did only 31 mothers (54 4%) and most of these part time only The economic situation was relatively good in most cases Still 51 families (87 9%) found the extra expenses for their child s diabetes as a moderate or great problem while 9 families (15 5%) declared that they could not afford the diabetic diet Financial support from the welfare office had been given to 11 families (19 0%) during the past 4 years

Knowledge The result of the knowledge test is shown in Fig 1 In spite of the low number of cases in some of the different subgroups the results are presented in % for the sake of comparison Thus 75% of the parents but only 38% of the teenagers reached the level considered to represent adequate knowledge There was a positive correlation between the knowledge of parents and that of their children ($r=0\ 37$ $p<0\ 01$) The parents knowledge was negatively correlated to late onset of diabetes in their children ($r=0\ 32$ $p<0\ 05$) Among patients more than 12 years knowledge was significantly correlated to positive attitudes ($r=0\ 52$ $p<0\ 005$)

Attitudes According to the interviews 52 patients (92 6%) were estimated to have predominantly positive attitudes towards the treatment of diabetes (Table 3) To the questions is there anything particular in the treatment that you dislike? 28 patients (42 0%) answered nothing , followed by the insulin injections in 12 cases (18 0%) The most readily accepted part of the treatment was physical exercise while the visits to the dia

Table 3 *Attitudes towards the treatment of diabetes based on scoring of interviews with 56 diabetic children*

Score	Patients >12 years old (n=37)			All patients (n=56)			
	<2 0	2 0–2 5	>2 5	<2 0	2 0–2 5	>2 5	Mean
Diet (content)	9	17	11	11	27	18	2 3
Diet (regularity)	1	20	16	2	31	23	2 5
Physical exercise	3	13	21	3	20	33	2 6
Urinalysis at home	7	18	12	9	30	17	2 4
Insulin injections	15	3	19	18	6	32	2 3
Visits to diabetic clinic	11	23	3	16	35	5	2 1
Regularity of daily life	8	20	9	8	33	15	2 4
Total treatment	3	26	8	4	38	14	2 4

Table 1 *Duration of diabetes*

	Years					Total
	3–6	7–10	11–14	Mean	S D	no
Boys	16	11	5	7.0	2.9	32
Girls	9	10	7	7.8	3.0	26
Total no	25	21	12	7.3	3.0	58

and the patient's knowledge about and attitudes towards diabetes are related to the degree of metabolic control.

MATERIAL

A group of 58 insulin diabetics, 6–17 years of age, was studied. They were all beyond the postinitial remission period and the duration of diabetes varied from 3 to 14 years (Table 1). The age at onset of diabetes varied between 1–13 years (Table 2). Puberty according to Tanner (36) was passed in 21 (36.2%), present in 13 (22.4%) and not yet attained in 24 cases (41.4%). All patients were regularly seen at the diabetic clinic of the pediatric department 4–8 times a year. Treatment consisted of insulin, regular physical exercise, a regulated diabetic diet and daily *glucosuria control*. The latter component, however, was not introduced in the therapeutic programme until in 1970, when also more intensive efforts were made in general towards metabolic normalization. Further details regarding the material have been published elsewhere (25).

METHODS

The *social situation* of the families was analysed through personal interviews and by the means of questionnaires regarding income, education and actual job. On the basis of this information the parents were classified into social groups 1–3 in accordance with official Swedish statistics (26). Supplementary information was obtained from the tax registries and from social welfare offices.

Knowledge about diabetes and its treatment was estimated in patients and their parents (one or both) through a written knowledge test including 7 questions on diabetic diet, 2 on physical exercise, 3 on insulin, 7 on urinalysis and 7 general questions on other items such as acute complications. The questions were similar to those used by other authors (3, 6, 10, 12). Some questions which were considered to be more important than others were awarded 6 points, whereas the other questions were given 4 points. Subjects who obtained more than 3/4 of the maximum possible points were considered to have "adequate" knowledge. The test was performed under supervision without any information given beforehand and access to literature was not allowed. Three children were excluded because of low age.

Attitudes towards the treatment were studied in two ways in all but two patients who were less than 7 years old. In collaboration with the department of sociology at the University of Linköping a standardized interview was constructed containing 52 questions related to the following seven items: Content of diet, regularity of diet, physical exercise, urinalysis at home, insulin injections, visits to the diabetic clinic and regularity of the daily activities. Each answer was classified as negative, neutral or positive, giving 1–3 points respectively, and for each of the seven items the patients got a mean score from 1 (very negative) to 3 (very positive). In addition to these interviews a special attitude test was constructed in collaboration with the department of Pedagogics at the University of Linköping. It consisted of 34 provocative statements related to the same items as the interviews. Each field was covered by negative and positive statements which the patients had to accept or reject, whereby the attitudes could be classified from very negative to very positive.

Psychological assessment of the patients was made through interviews and school contacts.

The quality of the diet, exercise and insulin treatment was determined during the spring 1975. *Diet* was estimated through a 24 hour recall performed by the dietitian and the results compared with the prescribed diet (?). In this way dietary habits could be classified as "good", "acceptable" or "poor". *Physical exercise* was recorded in detail by the patients or their parents during one week. According to the kind and duration of exercise the patients were classified in three categories: Very active, ordinarily active and inactive. *Insulin dose* (IU/kg body weight) and the number of insulin injections per day were taken as a measure of the insulin treatment.

The degree of *diabetic control* was determined for the whole of 1975 on the basis of the results of the patients' daily urinalysis by the Clinitest 2 drops method made at home. From the patients' diaries the number of tests showing less than 1% glucosuria was counted and expressed in percentage of all tests performed. This figure was taken as an index of diabetic control.

Laboratory methods. C peptide and insulin antibodies were determined as described elsewhere (5, 15, 25).

Statistical methods. Multiple regression analysis was the main technique employed. The following variables were included in the evaluation: age, (age)², age at onset, (age at onset)², duration, (duration)², onset after January 1, 1970, sex, stage of puberty, social group, income, knowledge about diabetes among parents, all patients and patients >12 years old, attitudes towards the treatment of the diabetes among all patients and among pa

Table 2 *Age at onset of diabetes*

	Years						Total
	1–2	3–6	7–10	11–13	Mean	S D	no
Boys	4	15	9	4	6.4	3.4	32
Girls	7	10	6	3	5.6	3.4	26
Total no	11	25	15	7	6.0	3.4	58

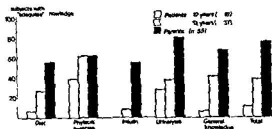


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Social situation The families belonged to social group I in 6 cases (10 3%) social group II in 18 cases (31 0%) and social group III in 34 cases (58 6%) The homes were broken in 5 cases Two patients lived in foster homes All fathers worked outside home but so did only 31 mothers (54 4%) and most of these part time only The economic situation was relatively good in most cases Still 51 families (87 9%) found the extra expenses for their child s diabetes as a moderate or great problem while 9 families (15 5%) declared that they could not afford the diabetic diet Financial support from the welfare office had been given to 11 families (19 0%) during the past 4 years

Knowledge The result of the knowledge test is shown in Fig 1 In spite of the low number of cases in some of the different sub groups the results are presented in % for the sake of comparison Thus 75% of the parents but only 38% of the teenagers reached the level considered to represent adequate knowledge There was a positive correlation between the knowledge of parents and that of their children ($r=0\ 37$ $p<0\ 01$) The parents knowledge was negatively correlated to late onset of diabetes in their children ($r=0\ 32$ $p<0\ 05$) Among patients more than 12 years knowledge was significantly correlated to positive attitudes ($r=0\ 52$ $p<0\ 005$)

Attitudes According to the interviews 52 patients (92 6%) were estimated to have predominantly positive attitudes towards the treatment of diabetes (Table 3) To the questions is there anything particular in the treatment that you dislike? 28 patients (42 0%) answered nothing followed by the insulin injections in 12 cases (18 0%) The most readily accepted part of the treatment was physical exercise while the visits to the dia

Table 3 *Attitudes towards the treatment of diabetes based on scoring of interviews with 56 diabetic children*

Score	Patients >12 years old ($n=37$)			All patients ($n=56$)			Mean
	<? 0	? 0–2 5	>? 5	<? 0	? 0–2 5	>2 5	
Diet (content)	9	17	11	11	27	18	2 3
Diet (regularity)	1	0	16	2	31	23	2 5
Physical exercise	3	13	21	3	20	33	2 6
Urinalysis at home	7	18	12	9	30	17	2 4
Insulin injections	15	3	19	18	6	32	2 3
Visits to diabetic clinic	11	23	3	16	35	5	2 1
Regularity of daily life	8	20	9	8	33	15	2 4
Total treatment	3	26	8	4	38	14	2 4

Table 1 *Duration of diabetes*

	Years					Total no
	3–6	7–10	11–14	Mean	S D	
Boys	16	11	5	7 0	2 9	32
Girls	9	10	7	7 8	3 0	26
Total no	25	21	12	7 3	3 0	58

and the patient s knowledge about and attitudes towards diabetes are related to the degree of metabolic control

MATERIAL

A group of 58 insulin diabetics 6–17 years of age was studied They were all beyond the postinitial remission period and the duration of diabetes varied from 3 to 14 years (Table 1) The age at onset of diabetes varied between 1–13 years (Table 2) Puberty according to Tanner (36) was passed in 21 (36 2%) present in 13 (22 4%) and not yet attained in 24 cases (41 4%) All patients were regularly seen at the diabetic clinic of the pediatric department 4–8 times a year Treatment consisted of insulin regular physical exercise a regulated diabetic diet and daily glucosuria control The latter component however was not introduced in the therapeutic programme until in 1970 when also more intensive efforts were made in general towards metabolic normalization Further details regarding the material have been published elsewhere (25)

METHODS

The *social situation* of the families was analysed through personal interviews and by the means of questionnaires regarding *income education and actual job* On the basis of this information the parents were classified into social groups 1–3 in accordance with official Swedish statistics (26) Supplementary information was obtained from the tax registries and from social welfare offices

Knowledge about diabetes and its treatment was estimated in patients and their parents (one or both) through a written knowledge test including 7 questions on diabetic diet 2 on physical exercise 3 on insulin 7 on urinalysis and 7 general questions on other items such as acute complications The questions were similar to those used by other authors (3 6 10 12) Some questions which were considered to be more important than others were awarded 6 points whereas the other questions were given 4 points Subjects who obtained more than 3/4 of the maximum possible points were considered to have adequate *knowledge* The test was performed under supervision without any information given beforehand and access to literature was not allowed Three children were excluded because of low age

Attitudes towards the treatment were studied in two ways in all but two patients who were less than 7 years old In collaboration with the department of sociology at the University of Linkoping a standardized interview was constructed containing 52 questions related to the following seven items Content of diet regularity of diet physical exercise urinalysis at home insulin injections visits to the diabetic clinic and regularity of the daily activities Each answer was classified as negative neutral or positive giving 1–3 points respectively and for each of the seven items the patients got a mean score from 1 (very negative) to 3 (very positive) In addition to these interviews a special attitude test was constructed in collaboration with the department of Pedagogics at the University of Linkoping It consisted of 34 provocative statements related to the same items as the interviews Each field was covered by negative and positive statements which the patients had to accept or reject whereby the attitudes could be classified from very negative to very positive

Psychological assessment of the patients was made through interviews and school contacts

The quality of the diet exercise and insulin treatment was determined during the spring 1975 *Diet* was estimated through a 24 hour recall performed by the dietitian and the results compared with the prescribed diet (?) In this way dietary habits could be classified as good acceptable or poor *Physical exercise* was recorded in detail by the patients or their parents during one week According to the kind and duration of exercise the patients were classified in three categories Very active ordinarily active and inactive *Insulin dose* (IU/kg body weight) and the number of insulin injections per day were taken as a measure of the insulin treatment

The degree of *diabetic control* was determined for the whole of 1975 on the basis of the results of the patients daily urinalysis by the Clinitest 2-drops method made at home From the patients diaries the number of tests showing less than 1% glucosuria was counted and expressed in percentage of all tests performed This figure was taken as an index of diabetic control

Laboratory methods C peptide and insulin antibodies were determined as described elsewhere (5 15 25)

Statistical methods Multiple regression analysis was the main technique employed The following variables were included in the evaluation age (age)^2 age at onset (age at onset)^2 duration (duration)^2 onset after January 1 1970 sex stage of puberty social group income knowledge about diabetes among parents all patients and patients >12 years old attitudes towards the treatment of the diabetes among all patients and among pa

Table 2 *Age at onset of diabetes*

	Years						Total no
	1–2	3–6	7–10	11–13	Mean	S D	
Boys	4	15	9	4	6 4	3 4	32
Girls	7	10	6	3	5 6	3 4	26
Total no	11	25	15	7	6 0	3 4	58

Table 5 *Multiple regression analysis of relation between the index of diabetic control and some clinical and laboratory variables in 57 patients*

	T	*r*	*p*
(*a*)			
Age	−3 63	−0 46	<0 001
(Age)	+3 31	+0 43	<0 00?
Duration	+4 01	+0 50	<0 001
(Duration)²	−3 98	−0 50	<0 001
Onset after Jan 1 19 0	+3 01	+0 40	<0 005
C peptide	+2 51	+0 34	<0 02
Insulin antibodies (IgG)	−3 01	−0 40	<0 005
Physical exercise	+4 45	+0 54	<0 001
(*b*)			
Dietary habits	+0 40	+0 06	n s
Knowledge about diabetes	−? 51	−0 35	<0 0_
Attitudes towards diabetes	−1 38	−0 20	n s
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variables were kept constant The correlation between knowledge and index of control was positive though weak and there was no significant correlation between social situation and degree of diabetic control When the variables in Table 5*b* were added to those in Table 5*a* there was only a weak correlation between dietary habits and the index of control Good knowledge among teenagers about diabetes was negatively correlated to the index of control (p<0 02) while the factor positive attitudes towards diabetes was weakly correlated to control However good knowledge *combined* with positive attitudes was positively correlated to the index of control (p<0 01)

DISCUSSION

As blood glucose represents a valid indicator of the metabolism and glucosuria can be assumed to reflect the blood glucose level rather well in young people with a normal renal threshold for glucose (1 8 14 18 29 31 41) daily testing of the urine for glucose was chosen as the most accurate way to estimate metabolic control

The social situation of the diabetic children in this study was comparable with that of other Swedish children according to official statistics (32) However in all but one case the parents had to take care of their diabetic child themselves which in several cases prevented the mother to take a job outside home Many parents also felt the economic burden of the diabetic treatment as a problem (9 39) In earlier studies a relationship between social problems and poor control has been found by some investigators (33 35) but not by others (20 42) In this study there was no significant correlation between social situation and degree of control but the quality of treatment was influenced as some families had obvious difficulties in purchasing a correct diet

In spite of regular and systematic information activities many of the parents and the majority of the diabetic children even above the age of 12 years (6 10 30) had unsatisfactory knowledge about diabetes and its treatment This is in agreement with other studies (3 6 10 12 19 40 42) Previous studies have not been able to demonstrate a correlation between knowledge and degree of control (6 11 19 34 37 40) Williams et al (42) even found a negative such relationship Their results probably reflect methodological problems and do not really contradict the common opinion that a good knowledge is essential for the management of juvenile diabetes The results of the present study seem to support the hypothesis that knowledge really has a positive effect on control and this effect was especially evident when knowledge was combined with positive attitudes

Many authors have found emotional problems more often in patients with poor diabetic control than in those with good control (13 20 33 35) This was also the case in this study Although emotional stress has a diabetogenic effect on the metabolism (16 17) the occurrence of emotional problems among poorly controlled young diabetics might as

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Psychological situation Intellectual capacity was within normal limits in all patients Severe psychological problems leading to psychiatric consultation had occurred in 7 cases These 7 patients all but one rebellious teenagers, showed more negative attitudes than other patients towards the treatment especially towards the diet and visits to the diabetic clinic and they had a lower index of control than the rest of the patients ($p<0\ 001$)

Diet Twenty nine patients (50 0%) followed their individually prescribed dietary plans containing the daily number of exchange portions of different categories of food The comparison between the results of the 24 hour recalls and the prescribed diets showed that 12 patients (20 7%) could be considered to have good food habits 35 patients (60 3%) an acceptable and 11 patients (19 0%) a poor diet However even those with a poor diet had a roughly regulated diet without concentrated carbohydrates Patients above the age of 12 years had less good food habits than the rest ($r=-0\ 35$ $p<0\ 02$) Among these older children however there was a trend towards better food habits among those with a better knowledge about the treatment of diabetes ($r=0\ 26$ $p<0\ 10$)

Physical exercise Fifteen patients (25 9%) were classified as being very active taking part in daily sports of some kind, 15 patients (25 9%) were inactive not taking part in any sports while the remaining 28 patients (48 3%) were classified as ordinarily active Age above 12 years was negatively correlated to physical exercise ($r=-0\ 41$ $p<0\ 005$) while a positive attitude towards treatment was correlated to the degree of exercise ($r=0\ 39, p<0\ 005$)

Insulin treatment Two injections a day were given to 24 patients (41 4%) while 34 patients (58 6%) had one injection a day All patients had a morning dose of longacting insulin (Novo Lente or Monotard) in 39 cases (62 7%) supplemented by intermediate or shortacting insulin (Novo Semilente or Actrapid) The mean insulin dose per kg body weight was 1 1 IU (range 0 6–1 8)

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Diabetic control could be assessed in 57 patients (98 3%) who tested their urine regularly In the majority of cases more than 50% of the tests showed less than 1% glucosuria (Table 4)

The partial correlations between index of control and some of the clinical and laboratory variables are shown in Table 5 Thus there was a non linear relationship between index of control age and duration ($p<0\ 002$) in such a way that the index of control gradually decreased up to the age of 14 years and then increased while in relation to duration the index gradually increased up to 10 years of duration but decreased thereafter Patients with onset after 1970 had a higher index of control ($p<0\ 005$) C peptide was positively correlated to the index of control ($p<0\ 02$) while the level of insulin antibodies (IgG) was negatively correlated to control ($p<0\ 005$) Physical exercise was the only part of the treatment that was positively correlated to diabetic control ($p<0\ 001$) when all other

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Submitted Sept 7 1976
Accepted Jan 21 1977

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well be a consequence of the poor control as the cause of it

There was a rather strong evidence of a positive influence of exercise on diabetic control The lack of correlation between the adherence to a prescribed diet and the degree of control does not contradict the importance of diet Our method to estimate dietary habits are too rough especially for finding significant differences in food habits between patients like ours who all have a more or less regulated diet However the results might suggest that a strict diet does not benefit diabetic control more than a moderately regulated diet if it is properly combined with regular physical exercise adequate insulin treatment and daily urinalysis (23 38) The positive effect of adequate insulin treatment on diabetic control does not need to be proven

As diabetic control is related not only to the quality of treatment but also to endogenous factors such as C peptide and insulin antibodies (24, 25) it was advisable to include these variables in the multiple regression analysis For the same reason it was necessary to include the factor onset after Jan 1 1970 as this factor reflecting an intensification of therapy has previously been shown to be of importance for the diabetic control in the same patients as in this study (25) The findings of a positive effect of C peptide and onset after 1970 and a negative effect of insulin antibodies on diabetic control were confirmed

In conclusion the results support the view that not only endogenous but also exogenous factors influence diabetic control Of the latter physical exercise was shown to be the most important The results further indicate an association between the social situation and the treatment and support the expected importance of the patients knowledge and attitudes for the therapeutic results In addition to the classical therapeutic tools in juvenile diabetes (insulin exercise and diet) more efforts should be made to improve the patients socio psychological adaptation to the strains of diabetic therapy

ACKNOWLEDGEMENTS

My sincere gratitude to my collaborators at the departments of Mathematics Pedagogics and Sociology at the University of Linkoping I am especially indebted to Mr Erik Leander Ph D and to Mr Bengt Berglov B A for their invaluable help and advice in doing the statistics My thanks also to Mrss Ketti Andersson Eva Cornell Britt Jeirud and Helene Nordahl for their skilful assistance

This work was supported by the Swedish Medical Research Council no B 75 19X-4528 01 by the Swedish Diabetic Association and by the Medical Faculty Linkoping University Sweden

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Submitted Sept 2 1976
Accepted Jan 21 1977

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BLOOD PRESSURE IN CHILDREN AND ADOLESCENTS

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ABSTRACT Cassimos Ch Varlamis G Karamperis S and Katsouyannopoulos V (Paediatric Clinic of Thessaloniki University Thessaloniki Greece) Blood pressure in children and adolescents Acta Paediatr Scand 66 439 1977.—Blood pressure (BP) levels were recorded in 2223 male and 2205 female children and adolescents ranging in age from 7 to 18 years In addition 521 male adults (soldiers) ranging in age from 21 to 25 years were included in the study Children and adolescents who participated in the survey were selected at random from the Elementary and High Schools The results of the study showed that a gradual increase occurred in the systolic as well as in the diastolic component of blood pressure from 7 to 18 years of age By contrast there was no increase with age in the systolic and diastolic blood pressure in the young male adult subjects who had BP measurements comparable to those observed in children A child was characterized as hypertensive according to the criteria outlined by Master et al Children with BP between the 90th and the 95th percentile were considered as suspect hypertensive whereas those with BP exceeding the 95th percentile were considered definitely hypertensive The overall incidence of hypertension in children in this survey was 3 1%

KEY WORDS Children adolescents hypertension blood pressure

There are relatively little data available in the pediatric literature regarding the level of arterial blood pressure in children (2 10 11 12) In Greece there is only one study published in a limited number of children relating blood pressure measurements to age The purpose of this study was two-fold First to obtain data regarding the distribution of blood pressure levels in different age groups in children and adolescents in Greece and compare them with those in young male adults Second to determine the incidence of hypertension in this population utilizing Master s criteria

MATERIAL AND METHODS

Children and adolescents attending the Elementary or High School as well as recruits from a large army unit (young male adult group) were included in the survey Blood pressure determinations were made in 2223 male children and adolescents 2 05 female children ranging in age from 7 to 18 years and 5?1 young male adults (soldiers) with an age of 21 to 25 years

The selection of the subjects was made as follows A certain number of elementary and high schools were picked at random from a list containing all the schools in the city of Thessaloniki Pupils attending these schools are by and large from families with an urban background Further selection of the subjects from the schools was made without any criteria from each school utilizing 1 in 3 pupils again randomly selected

In Greece elementary education for children aged 7–1? years is mandatory and approximately 95% of this population attend Elementary schools whereas 90% of adolescents attend High Schools

The selection of military recruits was made as follows One in 5 re ruits was selected at random without any predetermined criteria The origin of these recruits is representative of a cross-section population and represent urban as well as rural segments of the Greek community The socioeconomic status of the subjects was not taken into consideration

Blood pressure was recorded in the right arm by the same investigator after a 10 minute resting period A standard mercury sphygmomanometer Erkameter was utilized Depending on the arm size of the subjects two cuff sizes were used i e 9×37 and 13×39 cm² The reading on the sphygmomanometer which coincided with

Table 3 *Systolic and diastolic blood pressure measurements in young male adults according to age*

Data based on readings of 5'1 subjects

Age (y)	No of subjects	Systolic blood pressure Mean±S D (mmHg)	Systolic Percentiles 90th	Systolic Percentiles 95th	Diastolic blood pressure Mean±S D (mmHg)	Diastolic Percentiles 90th	Diastolic Percentiles 95th
21	111	124 41±11	138 9	147 5	77 23±7 '8	90 25	96 25
2'	152	123 26±10 58	138 43	139 73	77 91±8 15	91 7	96 14
23	88	120 34± 9 76	136 7	138 56	75 52±7 46	91 11	95 05
'4	110	1'3 89±10 6	133 05	141 5	76 98±7 83	89 97	95 45
25	60	124 83±11 8	138 47	140 25	79 ±8 79	98 8	102 56
Total	5'1						

ponent There was no statistically significant difference between the sexes in different age groups with the exception of the ages of 16–18 years where the mean systolic BP was higher in the male than in the female subjects ($p<0\ 005$) It was also of interest that the 95th percentile of the systolic component of BP was higher in the male than in female subjects *in the age group of 15–18 years* ($p<0\ 005$)

A gradual increase in the mean of systolic and diastolic BP as well as 90th and 95th percentiles was noted with age

Young male adults

There was no consistent pattern in the systolic and diastolic BP measurements relating to age in the young male adults (army recruits) as shown in Table 3

Elevated blood pressure

70 male subjects out of 2223 (3 14%) had *blood pressure levels which exceeded the 95th* percentile and were considered as definitely hypertensive In the female group 69 subjects out of 2205 (3 1%) had elevated blood pressure levels i e above the 95th percentile 52 4% of the 70 male subjects had systolic hypertension only 34 2% diastolic and 14 2% systolic and diastolic elevation of blood pressure 60 9% of 69 female subjects had systolic hypertension only 32 8% diastolic hypertension only and 5 8% systolic and diastolic (Table 4) Among 521 young male subjects 44 (8 4%) had systolic and/or diastolic blood pressure levels above the 90th percentile and 17 (3 3%) *above the 95th percentile for age* (Table 5)

Table 4 *Incidence and types of hypertension in children and adolescents aged 7 to 18 years*

	Males	Females
Total number studied	2 223	2 205
Number and percentage with hypertension	70 (3 1%)	69 (3 1%)
Number and percentage with		
Systolic hypertension	36 (51 4%)	4? (60 8%)
Diastolic hypertension	24 (34 2%)	23 (32 8%)
Systolic and diastolic hypertension	10 (14 2%)	4 (5 8%)
Number and percentage of suspect hypertensive subjects	136 (6 1%)	110 (5 0%)

'9 77 874

DISCUSSION

It is generally accepted that the determination of blood pressure in children is often neglected

Table 5 *Incidence and types of hypertension in young male adults*

Total number of subjects studied	521
Number and percentage of subjects with hypertension	17 (3 3%)
Number and percentage of subjects of suspect hypertension	27 (5 ?%)
Number and percentage with	
Systolic hypertension	13 (6 ?%)
Diastolic hypertension	– –
Systolic and diastolic hypertension	4 (23 8%)

Table 1 *Systolic blood pressure measurements according to age*
Data based on readings in 2 223 male and 2 205 female children and adolescents

Age (y)	No of subjects		Mean ±S D (mmHg)		Percentiles			
					Males		Females	
	Males	Females	Males	Females	90th	95th	90th	95th
7	163	124	108 23±10 14	107 93± 8 55	125 6	128 2	124 5	128
8	162	156	109 18± 9 26	110 43± 8 73	125 5	128 2	126 5	128 6
9	230	194	110 29± 9 88	110 63± 9 73	125 4	128 4	127 1	129 1
10	208	251	114 63± 9 57	115 15±10 16	1?8 1	132 4	132	136 6
11	186	192	114 21± 9 6	117 56± 9 35	128 6	129 8	133 6	137 5
12	207	216	117 2 ± 8 39	118 53±10 17	129 4	133 5	136 3	138 4
13	180	185	114 68±19 08	115 69±11 21	129	132 4	134	137 2
14	176	214	117 66±11 36	118 65±10 96	134 2	137 4	135 2	139 2
15	181	162	118 51±10 49	119 4 ±10 16	133 2	137	135 6	138 7
16	167	126	122 26±10 52	1?0 83± 9 82	138 2	141	136 2	138 8
17	170	189	121 09± 9 55	119 89±11 4	136 7	140	136 2	139 8
18	19?	196	122 83±10 68	117 01±10 81	139	144	134	137 5
Total	2 22?	2 205						

the appearance of the pulse sound was recorded as the systolic component of the blood pressure whereas the reading corresponding to the complete disappearance of the sound was recorded as the diastolic component (5 8 10 11 12 18 19)

Blood pressure levels were considered elevated when they exceeded the 95th percentile In the event that the systolic and/or diastolic component were elevated blood pressure measurements were repeated a week later under similar conditions and the lowest value was considered as the final level of blood pressure The reading of the *blood pressure was done on the sphygmomanometer scale* with each division corresponding to 2 mmHg

The mean standard deviation and percentiles for each *of the three groups as well as the computation of BP* measurements in each group were calculated at the Computing Center University of Thessaloniki

RESULTS

Children and adolescents

The data of the systolic component of the blood pressure according to age and sex as well as the percentiles are shown in Table 1 Table 2 shows the results of the diastolic com

Table 2 *Diastolic blood pressure measurements in children and adolescents according to age*

Age (y)	No of subjects		Mean ±S D (mmHg)		Percentiles			
					Males		Females	
	Males	Females	Males	Females	90th	95th	90th	95th
7	163	124	72 87±10 25	72 64± 8 22	87 9	89 9	87 6	89 3
8	162	156	73 65± 9 89	73 99± 9 8	88 9	91 9	89 8	95 2
9	230	194	74 55± 9 01	73 6 ± 9 61	88 9	92 4	89 5	94
10	208	251	75 54±10 36	73 31±10 08	89 7	95	89	93
11	186	192	74 82± 8 52	74 22± 9 46	89 1	9? 6	89 2	92 3
12	207	216	75 54± 8 58	75 81± 8 57	89 1	92 8	89 5	94 8
13	180	185	73 57± 8 67	74 39± 9 65	88 4	90	90	95
14	176	214	73 27± 9 9	75 72± 9 16	88 9	91 9	90 8	95 6
15	181	162	74 19± 9 52	77 59± 9 09	89 1	92 8	94	95
16	167	1?6	76 92± 8 59	77 91± 9 47	90 8	95 4	89 9	92 2
17	170	189	77 31± 8 78	77 83± 9 4	92 8	96 8	93 6	97 2
18	19?	196	76 93± 9 18	77 67± 9 13	92 2	96 3	94 6	97 4
Total	2 223	2 20?						

Table 3 *Systolic and diastolic blood pressure measurements in young male adults according to age*

Data based on readings of 521 subjects

Age (y)	No of subjects	Systolic blood pressure Mean±S D (mmHg)	Percentiles 90th	95th	Diastolic blood pressure Mean±S D (mmHg)	Percentiles 90th	95th
21	111	124 41±11	138 9	147 5	77 23±7 28	90 25	96 25
	152	123 76±10 58	138 43	139 73	77 91±8 15	91 7	96 14
23	88	120 34± 9 76	136 7	138 56	75 52±7 46	91 11	95 05
24	110	123 89±10 6	133 05	141 5	76 98±7 83	89 97	95 45
25	60	124 83±11 8	138 47	140 25	79 ±8 79	98 8	102 56
Total	521						

ponent There was no statistically significant difference between the sexes in different age groups with the exception of the ages of 16–18 years where the mean systolic BP was higher in the male than in the female subjects ($p<0\ 005$) It was also of interest that the 95th percentile of the systolic component of BP was higher in the male than in female subjects in the age group of 15–18 years ($p<0\ 005$)

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Table 4 *Incidence and types of hypertension in children and adolescents aged 7 to 18 years*

	Males	Females
Total number studied	2 223	2 205
Number and percentage with hypertension	70 (3 1%)	69 (3 1%)
Number and percentage with		
Systolic hypertension	36 (51 4%)	42 (60 8%)
Diastolic hypertension	24 (34 2%)	23 (32 8%)
Systolic and diastolic hypertension	10 (14 2%)	4 (5 8%)
Number and percentage of suspect hypertensive subjects	136 (6 1%)	110 (5 0%)

Elevated blood pressure

70 male subjects out of 2223 (3 14%) had blood pressure levels which exceeded the 95th percentile and were considered as definitely hypertensive In the female group 69 subjects out of 2205 (3 1%) had elevated blood pressure levels i e above the 95th percentile 52 4% of the 70 male subjects had systolic hypertension only 34 2% diastolic and 14 2% systolic and diastolic elevation of blood pressure 60 9% of 69 female subjects had systolic hypertension only 32 8% diastolic hypertension only and 5 8% systolic and diastolic (Table 4) Among 521 young male subjects 44 (8 4%) had systolic and/or diastolic blood pressure levels above the 90th percentile and 17 (3 3%) above the 95th percentile for age (Table 5)

DISCUSSION

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Table 5 *Incidence and types of hypertension in young male adults*

Total number of subjects studied	521
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Table 1 *Systolic blood pressure measurements according to age*
Data based on readings in 2 223 male and 2 205 female children and adolescents

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10	208	251	114 63± 9 57	115 15±10 16	128 1	132 4	132	136 6
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15	181	162	118 51±10 49	119 4 ±10 16	133 2	137	135 6	138 7
16	167	126	122 26±10 52	120 83± 9 82	138 2	141	136 2	138 8
17	170	189	121 09± 9 55	119 89±11 4	136 7	140	136 2	139 8
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Blood pressure levels were considered elevated when they exceeded the 95th percentile In the event that the systolic and/or diastolic component were elevated blood pressure measurements were repeated a week later under similar conditions and the lowest value was considered as the final level of blood pressure The reading of the blood pressure was done on the sphygmomanometer scale with each division corresponding to 2 mmHg

The mean standard deviation and percentiles for each of the three groups as well as the computation of BP measurements in each group were calculated at the Computing Center University of Thessaloniki

RESULTS

Children and adolescents

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Table 2 *Diastolic blood pressure measurements in children and adolescents according to age*

Age (y)	No of subjects		Mean ±S D (mmHg)		Percentiles			
					Males		Females	
	Males	Females	Males	Females	90th	95th	90th	95th
7	163	124	72 87±10 25	72 64± 8 22	87 9	89 9	87 6	89 3
8	162	156	73 65± 9 89	73 99± 9 8	88 9	91 9	89 8	95 2
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14	176	214	73 27± 9 9	75 72± 9 16	88 9	91 9	90 8	95 6
15	181	162	74 19± 9 52	77 59± 9 09	89 1	92 8	94	95
16	167	126	76 92± 8 59	77 91± 9 47	90 8	95 4	89 9	92 2
17	170	189	77 31± 8 78	77 83± 9 4	92 8	96 8	93 6	97 2
18	193	196	76 93± 9 18	77 67± 9 13	92 2	96 3	94 6	97 4
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Submitted July 12 1976
Accepted Jan 21 1977

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as a necessary component of the physical examination This is due to following reasons first the difficulty related to accurate recording, second the notion that idiopathic hypertension does not constitute a problem in childhood and third the lack of established normal values in different age groups for comparison (1 2 10, 11 14 21) There are few studies currently available and the data presented in these studies are not based on uniform criteria (4 10 11 15 19) More recently two surveys were published by Londe (11) and by Weiss et al (21) on 1473 and 7119 subjects respectively which were conducted in a systematic fashion

It is possible that epidemiological studies in children will shed some light on the natural history of hypertension in adults It may be that essential hypertension first appears in childhood possibly before the age of three years (19 20) Several workers have shown that a correlation exists between childhood hypertension and obesity as well as with the family history of essential hypertension in adults (11 12 23)

Deschamps et al (3) have reported an increased incidence of cerebral and cardiovascular accidents in family members of children with elevated blood pressure at the age of 4 to 6 years (3) The difference in blood pressure in the male subjects at the age of 16–18 years in our study confirms previous observations (12 13 20 21) Our data also agree with those from other surveys (11 12 19 21) regarding mean systolic blood pressure the values of 90th and 95th percentiles and the yearly increment of the systolic component of blood pressure However the mean diastolic blood pressure as well as the 90th and 95th percentiles were relatively higher in our study The average yearly increment in the diastolic blood pressure in our study was 3 mmHg which is comparable with that reported in the literature (21 22)

In the young male adult group the mean values as well as the 90th and 95th percentiles of the systolic and diastolic blood pressure neither increased with age, nor differed considerably from the corresponding values in the age group of 17–18 years It appears, therefore that blood pressure levels off between the 16th and 18th year and no further rise is observed in the male group until the age of 21 years Our findings agree with those reported in the literature (13 21)

A child was characterized as hypertensive according to Master s criteria (16) which were also utilized by Londe (11) Subjects with blood pressure between the 90th and 95th percentile were considered as suspect hypertensive while subjects with blood pressure above the 95th percentile were considered definitely hypertensive The incidence of childhood hypertension in this study was 3 1% which is in agreement with the studies of Londe (11) and Lagos et al (9)

The group of children considered as definitely hypertensive i e above the 95th percentile are currently under investigation and the findings of this study will be reported in due course

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Submitted July 1? 1976
Accepted Jan 21 1977

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Acta Pædiatr Scand 66 445–449 1977

ENDOCRINE ASPECTS OF TRISOMY 4p

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ABSTRACT Giovannelli G Bernasconi S and Forabosco A (Department of Paediatrics Parma and Institute of Human Anatomy Modena Italy) Endocrine aspects of trisomy 4p Acta Paediatr Scand 66 445 1977 —The main endocrinological parameters were investigated in two sisters affected with trisomy 4p Our findings rule out any impairment of the endocrine system in this rare syndrome even in those cases in which stunting of growth is more pronounced The marked weight deficit in one of the two patients had no relationship to the chromosomal anomaly it was determined by the association of a deficit of immunoglobulin with a Giardia Lamblia infestation

KEY WORDS Trisomy 4p stunted growth Giardia Lamblia infestation immunoglobulin deficiency

Trisomy of the short arm of chromosome 4 (4p+) is a newly recognized syndrome (1) characterized by mental retardation and stunted growth skeletal dysmorphism with prevalent midline distribution (2) and characteristic facial morphology

The phenotypic as well as the radiological patterns have already been described in detail (3) Stunting of growth is of variable degree out of 10 cases found in literature the height of 5 was ≤the 3rd percentile (2 9 10 11) in 3≤ the 10th percentile (12 13 14) and in 2≤the 25th percentile No endocrinological assessment of this syndrome has yet been obtained the purpose of the present paper is to fill this gap with data collected from two sisters (fig 1) in which the trisomy 4p due to maternal translocation *t* (4 22) (p11 p12) (Fig 2) has previously been documented (4)

PATIENTS AND METHODS

Case 1 M S 12 10/12 years Bone age 11 yrs by the Greulich and Pyle method Height 132 8 cm (<3rd percentile) Weight 25 2 kg (<3rd percentile)

Case 2 M N 6 4/12 years Bone age 6 years by the Greulich and Pyle method Height 111 1 cm (25th percentile)

Both cases (Fig 3) show the classical phenotype of the trisomy 4p short stature microcephaly broad thorax with widely spaced nipples prominent supraorbital margins forming a horizontal crest with the glabella hypertelorism antimongoloid slanted eyes broad bridged nose with bulbous fleshy tip mouth malocclusion low set malformed ears Radiological findings common to both patients include microcephaly small sella turcica mandibular hypoplasia with obtuse mandibular angle protrusion of the upper incisors marked hypoplasia of the first rib Y shaped configuration of clavicles right-convex scoliosis straightening of the spine in lateral projection due to absence of the physiological curvatures increase of the iliac and flattening of the acetabular angles rectangular shape of iliac crests and bilateral coxa valga

In case 2 the middle phalanges of both hands show a bowling pin appearance due to flaring of the metaphyses In case 1 small bowel examination shows the radiological pattern of a malabsorption syndrome This is related to a severe prolonged diarrhea which suddenly began at age 11 6/12 years

Intestinal biopsy (Fig 4) demonstrated a malabsorption syndrome of epithelial type with normal pancreatic secretion Schilling test=8 4% (n v ≈10–35%) Xylose test= 13 5% (n v =15%) fecal fat=5 3 g/24 h (n v =5 g/24 h) The malabsorption probably arose from a combination of a Giardia Lamblia infestation and serum immunoglobulin deficiency (IgG=355 mg/100 ml IgM=10 mg/100 ml IgA=34 mg/100 ml) Therapy directed against the Giardia

ENDOCRINE ASPECTS OF TRISOMY 4p

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PATIENTS AND METHODS

Case 1 M S 12 10/12 years Bone age 11 yrs by the Greulich and Pyle method Height 137 8 cm (<3rd percentile) Weight 25 7 kg (<3rd percentile)

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Fig 1 Karyotype of the proband (case 1) 46XX der (22) *t* (4 22) (p11 p12) *R* banding method
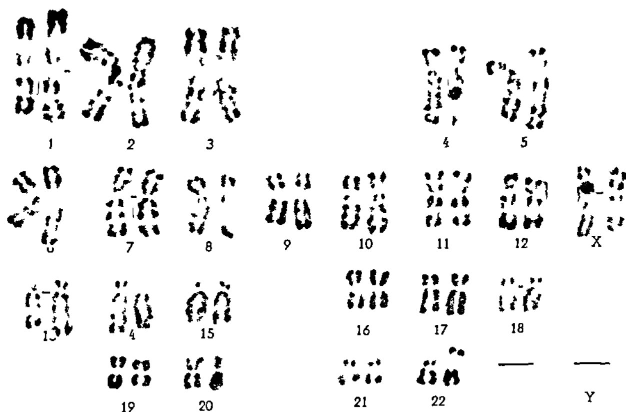


Fig 2 Karyotype of the mother with a translocation *t* (4 22) (p11 p12) *R* banding method



Fig 3 (*a*) Case 1 (M S 1? 10/12 yrs) (*b*) Case 2 (M N 6 4/12 yrs)

infestation (Furoxone® 100 mg T I D for 15 days) gave good results with the child putting on 4 kg in 3 months after almost 2 years of stationary weight

Endocrinological investigations performed are outlined in Table 1 For details see our previous publications (7 8)

RESULTS

While referring to Tables 2 and 3 for details the results can be summarized as follows

Pituitary reserve of GH Normal in both cases

Hypothalamus – pituitary – gonad axis Both FSH and LH plasma levels fall within



Fig 4 Intestinal biopsy The villi have disappeared at many points or are markedly flattened the interstitium shows oedema with much lymphatic infiltration the crypts are tortuous and elongated (H E ×30)

normal prepubertal range before and after LH RH stimulation in both cases

Hypothalamus – pituitary – adrenal axis This was investigated in case 1 only Basal secretion of 17 ketosteroids=1 24 and of 17 ketogenic steroids=5 9 mg/24 hrs with metyrapone administration the former increased to 5 4 and the latter to 43 2 mg/24 hrs Plasma cortisol levels increased regularly after ITT Altogether the functioning of the tested axis appears to be within normal limits

Hypothalamus – pituitary – thyroid axis In both cases basal values and pituitary reserve of TSH were normal as shown by the TRH stimulation test T_4 concentrations (8 5 μg/100 ml in case 1 and 7 5 μg/100 ml in case 2) T_3 test values (34 58% and 33 0%) and T_T-index values (3 11 and 2 48) were also within normal limits

Table 1 *Technical outline of the endocrinological investigations*

Hormones	Method	Material	Reference
LH and FSH	RIA—double antibody	Kit of Serono®	–
Cortisol	Fluorimetric	–	(5)
Insulin	RIA double antibody	Kit of CEA SORIN®	(8)
TSH	RIA double antibody	Kit of Serono®	–
T	RIA protein binding	Kit of Abbott®	–
T	RIA protein binding	Kit of Abbott®	–
GH	RIA double antibody	Kit of CEA SORIN®	(7)

Table 2 *Hormonal pattern and blood sugar behaviour in case 1*

Tested substances	Stimulus	Doses	Times of blood sampling (min) 0	2	10	15	20
GH (ng/ml)	Insulin i v	0 1 U/kg	0 7	–	–	–	–
Cortisol (μg/100 ml)	Insulin i v	0 1 U/kg	9 5	–	–	–	–
Blood sugar (g/l)	Insulin i v	0 1 U/kg	0 8	–	–	–	–
GH (ng/ml)	Arginine i v	0 5 g/kg	2 5	–	–	21 0	–
TSH (μU/ml)	TRH i v	200 μg/1 73 m²	4 2	–	22 6	–	22 2
LH (mU/ml)	LH RH i v	100 μg/1 73 m²	1 5	–	6 7	8 5	10 0
FSH (mU/ml)	LH RH i v	100 μg/1 73 m²	8 9	–	10 5	10 7	1? 5
Insulin (μU/ml)	Glucose p o	50 g/m²	29 7	–	–	53 0	–
Blood sugar (g/l)	Glucose p o	50 g/m²	0 8	–	–	1 4	–
Insulin (μU/ml)	Glucose i v	0 5 g/kg	18 0	64 0	–	29 3	–
Blood sugar (g/l)	Glucose i v	0 5 g/kg	0 9	4 1	–	2 3	–

However in case 1 the thyroid uptake of ¹³¹I was low 6% after 6 hours and 10% after 24 hours In the absence of other abnormal clinical and laboratory findings (scanning with ¹³¹I also showed a normal shaped gland with regular distribution of radioactivity) this abnormally low uptake has been considered due to a higher blood level of inorganic iodine resulting from i v pyelography performed about 1 month previously

β pancreatic function Normal in case 1 as demonstrated by plasma insulin levels after IVGTT and OGTT as well as by blood sugar levels after OGTT (6) it was not tested in case 2

CONCLUSION

No endocrine dysfunctions were demonstrated in the two patients with trisomy 4p Considering the small number of ascertained cases described up to now absence of endocrine impairment is likely to be the rule in this new syndrome Therefore even when stunting of growth is remarkable as in our case 1 an associated deficit of GH or thyroid hormones need not be considered the cause The marked weight deficit observed (in case 1) was clearly related to a malabsorption syndrome arising from the association of an immunoglobulin deficit with Giardia Lamblia infestation in fact there was rapid recovery after suitable therapy The above mentioned immunological deficit has been interpreted as an occasional transient finding not correlated with the chromosomal anomaly

ACKNOWLEDGEMENT

We are greatly indebted to Drs M Miglioli and P Vezzadini (Clinica Medica University of Bologna) for performing the intestinal biopsy and related investigations

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Table 3 *Hormonal pattern in case 2*

Tested hormones	Stimulus	Doses	Time of blood sampling (min) 0	10	15	20	30	45	60	90	120
GH (ng/ml)	Arginine i v	0 5 g/kg	4 6	–	4 0	–	11 0	–	8 3	7 3	1 5
TSH (μU/ml)	TRH i v	200 μg/1 73 m²	4 2	19 3	–	18 0	14 4	–	11 0	5 6	4 8
LH (mU/ml)	LH RH i v	100 μg/1 73 m²	1 5	1 8	2 4	3 3	3 0	–	3 8	–	1 6
FSH (mU/ml)	LH RH i v	100 μg/1 73 m²	2 8	5 3	4 8	6 3	8 0	–	11 0	–	8 5

30	45	60	90	120	180
3 3	–	10 0	–	18 0	–
	–	31 0	31 5	36 3	–
0 5	–	0 5	0 4	0 6	–
15 8	6 80	3 80	3 0	3 30	–
22 6	–	15 8	6 8	5 8	–
13 0	–	12 0	–	6 30	–
15 5	–	16 0	–	17 0	–
83 0	64 0	35 3	36 5	14 0	11 0
1 5	1 5	1 2	0 8	0 9	0 9
25 0	–	15 5	–	19 0	–
1 7	–	0 9	–	0 6	–

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Submitted Sept 29 1975
Accepted Dec 28 1976

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Acta Pædiatr Scand 66 451–456 1977

CONTINUOUS NEGATIVE CHEST WALL PRESSURE THERAPY FOR ASSISTING VENTILATION IN OLDER CHILDREN WITH PROGRESSIVE RESPIRATORY INSUFFICIENCY[1]

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ABSTRACT *Sanyal S K Avery T L* Thapar M K Hughes W T and Harris K S (Cardiopulmonary Disease Service Intensive Care Unit and Infectious Disease Service St Jude Children s Research Hospital Memphis Tennessee USA) Continuous negative chest wall pressure therapy for assisting ventilation in older children with progressive respiratory insufficiency Acta Paediatr Scand 66 451 1977.—Continuous negative chest wall pressure (CNP) was used to assist ventilation in 14 children 6 months to 14 years of age who had progressive respiratory insufficiency caused by diffuse bilateral alveolar disease Before the start of CNP therapy each child had a respiratory rate>50/min arterial oxygen tension (PaO_2)<70 mmHg ($FIO_2 \geq 50\%$) and arterial carbon dioxide tension ($PaCO_2$)<45 mmHg The mean intrapulmonary right to-left shunt was 28 7±3 8% Within 6 hours after therapy was started PaO_2 increased from 55 4±15 9 to 81 6±17 7 mmHg ($p<0\ 005$) This improvement was sustained and within 24 hours permitted a decrease in fractional concentration of inspired oxygen (FIO_2) from 51 8±6 2 to 41 0±8 4% ($p<0\ 001$) and in respiratory rate from 78 1±23 0 to 56 4±21.3 ($p<0\ 01$) There was a concomitant decrease in intrapulmonary right to-left shunt Four of the 14 patients developed pneumothorax that was successfully decompressed Ten patients survived

These observations establish CNP therapy as an effective means of improving arterial oxygenation in spontaneously breathing older children Of added significance this mode of therapy eliminates the need for endotracheal intubation and prolonged use of muscle relaxants and sedatives It also minimizes exposure to high FIO_2 thereby minimizing the hazards of pulmonary oxygen toxicity

KEY WORDS Older children continuous negative pressure respiratory insufficiency arterial oxygenation

The effectiveness of continuous negative chest wall pressure (CNP) as a means of improving arterial oxygenation is now well established in newborn infants with severe respiratory distress syndrome (2 6 14) In a recent preliminary communication (17) we suggested that CNP therapy may prove to be equally effective in management of progressive hypoxemia due to extensive lung disease in spontaneously breathing older children The purpose of this communication is to present data that establish the role of CNP therapy as a means for assisting ventilation in spontaneously breathing older children who develop progressively severe hypoxemia of pulmonary

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[1] Part of these data were presented at the Annual Meeting of the American Thoracic Society and American Lung Association at New Orleans May 1976

Abbreviations CNP=continuous negative pressure PaO =arterial oxygen tension $PaCO_2$=arterial carbon dioxide tension RF=respiratory frequency FIO_2=fractional inspired O concentration QS/QT=intrapulmonary right to-left shunt FRC=functional residual capacity PCP=*Pneumocystis carinii* pneumonitis

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Table 1 *Comparison of vital signs and blood gas profile before during and after CNP therapy*

	On admission	Pre CNP	During CNP 1 hr	2 hr	3 hr	6 hr
CNP (cm H_2O)			5 9± 2 1	7 3± 3 3	9 6± 3 0	10 3± 2 7
PaO_2 (mmHg)	49 8±17 1	55 4±15 9	74 4±35 2	72 3±22 2	75 6±24 0	81 6±17 7
Respiratory frequency (per min)	56 6±23 0	78 1±23 0	66 7±23 1	64 9±26 9	61 9±24 2	58 1±20 3
FIO_2 (%)	Room Air	51 8± 6 2	51 4± 6 3	51 5± 7 2	50 8± 8 0	49 1± 8 1
Heart rate (per min)	*130 6±19 3*	*136 8±20 1*	*136 3±27 6*	*132 8±24 2*	*133 5±26 5*	*126 4±22 1*
(QS/QT)×100 (%)[c]	20 8± 8 6	28 7± 3 8	26 0± 3 0	–	–	–

[a] Mean±S D of 14 patients [b] Means±S D of 10 survivors [c] Intrapulmonary right to-left shunt

origin and to discuss briefly the basic mechanism that might explain the improvement of arterial oxygenation produced by this mode of therapy

MATERIALS AND METHODS

Patients

The study included 14 of 34 immunosuppressed children who while receiving chemotherapy for childhood malignancies were admitted to St Jude Children s Research Hospital with progressive bilateral alveolar disease due to *Pneumocystis carinii* infection Their ages ranged from 6 months to 14 years with median of 7 years Each patient had a history of fever cough and respiratory distress of 6 to 16 days duration

Physical examinations on admission showed mild to severe respiratory distress in all patients with respiratory rates ranging from 28 to 92 per min Heart rates varied from 110 to 170 per min The lungs of each child were clear to auscultation and percussion Eleven patients were febrile and one had cyanosis

Initial laboratory data included hemoglobin values ranging from 82 to 100 g/l and total white blood cell counts from (6 8–8 2×10⁹/l) *with a predominance of neutrophils* Urine and blood cultures were sterile and throat cultures were free of pathogenic organisms Skin tests for histoplasmosis and tuberculosis were nonreactive Complement fixation titers for influenza viruses A B and C parainfluenza viruses toxoplasma and mycoplasma were all less than 1 8 Chest roentgenograms showed bilateral pulmonary densities compatible with diffuse alveolar disease

Initial acid base and blood gas profiles (Table 1)

An analysis of arterial blood samples (18 19) obtained from 13 patients *while they were breathing room air indicated* moderate to severe hypoxemia (mean arterial oxygen tension (PaO_2) 49 8±17 1 mmHg range 27 to 80 mmHg) *Acid base measurements were normal in 3 patients* while the remaining 10 had respiratory alkalosis characterized by pH≥7 5 and arterial carbon dioxide tension ($PaCO_2$)≤30 mmHg

Arterial blood gas measurements were repeated in 14 patients after they had breathed 100% oxygen for 20 min *from a nonbreathing system equipped with a Hans* Rudolph valve and an in line reservoir Before each blood gas determination the oxygen electrodes were calibrated *with gas of known oxygen tension Alveolar arterial gradients* and intrapulmonary right to-left shunts were calculated from standard formulas (3 15 19) The results *indicated abnormal intrapulmonary right to-left shunts in* all but one patient (range of values 7 to 33%)

Hospital course

After the initial acid base and blood gas studies we obtained lung aspirates from each patient by a percutaneous transthoracic approach (11 12) These contained *P carinii* organisms as demonstrated with toluidine blue O and methenamine silver nitrate stains Cultures of the aspirates were free of bacteria and fungi Either pentamidine isethionate[1] or trimethoprim sulfamethoxazole (11) as well as chlorpheniramine intravenous fluids and oxygen were administered to all patients During the next 48 to 96 hours each patient developed progressive respiratory distress and hypoxemia that necessitated an increase in fractional inspired oxygen concentration (FIO_2) from room air to 51 5±7 2% There was a concomitant increase in intrapulmonary right to-left shunt from 20 8±8 6% on admission to 28 7±3 8% Because of progressive respiratory *insufficiency and the inability to maintain PaO_2≥70* mmHg in spite of an increase in FIO_2 to ≥50% CNP therapy was started for each patient The specific criteria *for starting therapy are shown in* Table 2

CNP therapy

A modified Emerson tank respirator (17) was used to apply continuous negative pressure around the chest wall and lower part of the body Blood gases were measured serially while the pressure inside the respirator was adjusted from −1 to −18 cm H_2O The negative pressure that resulted in a PaO_2>70 mmHg was maintained When two consecutive PaO_2 values of 70 mmHg or greater were obtained 1 hour apart *FIO_2 was reduced by decrements of* 5% until 40 to 35% was reached At this point CNP was reduced by decrements of 1 to 2 cm H_2O on the basis of

[1] Pentamidine isethionate investigational drug Trimethoprim sulfamethoxazole Bactrim Septra Chlorpheniramine Chlor Trimeton Cloropiral Polaronil Allergisan Chlor Tripolon Teldrin Histadur Piriton

2 hr	24 hr	48 hr	Post CNP[b]
11 2± 2 7	10 6± 3 6	8 7± 5 6	
77 0±19 7	73 8±16 5	84 8±25 7	86 5±25 1
60 5±21 9	56 4± 1 3	50 9±17 7	37 9±13 4
45 4± 6 9	41 0± 8 4	43 7±16 7	33 3± 6 1
27 0±21 9	123 9±22 4	127 5±18 2	106 8±21 0
–	24 4± 2 2	23 0± 1 5	16 4± 5 5

serial blood-gas measurements that were repeated within 1 hour after each change in either FIO or negative pressure When PaO_2 remained at or above 70 mmHg (with FIO of ≤40%) for 2 hours without CNP the patient was removed from the tank respirator and FIO_2 was gradually reduced as tolerated to that of room air

RESUL1S

For these 14 patients the duration of CNP therapy ranged from 1 5 to 15 days (mean 6 68±5 26) Therapy was started at a negative pressure of 5 cm H_2O for 11 patients 8 cm H_2O for two and 12 cm H_2O for one child who was considered to have severe ventilatory insufficiency characterized by cyanosis marked respiratory distress PaO_2 of 27 mmHg and $PaCO_2$ of 28 mmHg In an effort to distinguish the beneficial effects of CNP therapy from those possibly produced by improvements in the lung disease itself we analyzed in greater detail those changes occurring during the first 24 hours of therapy

Amount of continuous negative pressure

During the first 12 hours of therapy the amount of CNP increased as a curvilinear

Table 2 *Criteria for starting CNP therapy*

Age (y)	Respiratory rate (per min)	PaO_2 (mmHg)	Intrapulmonary right to-left shunt (%)
1– 5	>80	<60	>24
5–10	>65		
>10	>50		

Despite increases in FIO_2 to ≥50%

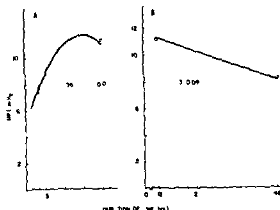


Fig 1 Kinetics of CNP during first 48 hours of therapy During the first 12 hours the amount of negative pressure was increased as a curvilinear function of time Note that the maximum increase in CNP occurred between 11 and 12 hours of therapy The amount of CNP was then decreased by 48 hours as a linear function of time

function of time reaching its maximum value between 11 and 12 hours (Fig 1) Increases in pressure during this period were positively correlated with cha iges in PaO_2 ($p<0\ 025$) and negatively with decreases in FIO_2 and respiratory frequency ($p<0\ 005$) After 12 hours CNP decreased linearly with time

Arterial oxygen tension

Within 1 hour after the start of CNP therapy absolute PaO_2 values increased in all patients but one (Fig 2) Similarly the mean PaO_2 increased from 55 4±15 9 before therapy to 74 4±35 2 within 1 hour By the end of 6 hours the increase in mean PaO was significantly different from the pre CNP mean ($p<0\ 005$) This improvement in arterial ox)genation although not linearly related to the amount of negative pressure persisted despite a continuous decrease in FIO_2 (Table 1)

Fractional concentration of inspired oxygen

During the first 2 hours of CNP therapy while negative pressure increased from a mean of 5 93±2 1 to 7 3±3 3 cm H_2O there was little change in mean FIO_2 From the 3rd to the 12th hour however the decrease in FIO_2 showed a significant negative correlation with the in

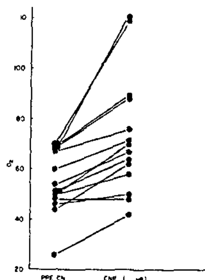


Fig 2 Effect of 1 hour of CNP therapy on arterial oxygenation in 14 patients In each patient the fractional concentration of inspired oxygen (FIO_2) and the amount of negative pressure were maintained at a constant level from the pre *CNP* period to *1* hour after therapy was begun

crease in amount of negative pressure that was applied around the chest wall ($p<0\ 005$) During this 9 hour period in which negative pressure increased from 9 6±3 0 to its maximum mean value of 11 2±2 7 cm H_2O it was possible to reduce FIO_2 by 3 46% for each increase in CNP of 1 cm H_2O By the end of 12 hours the mean FIO_2 values had decreased from a pre CNP high of 51 8±6 2% to 45 5±6 9% ($p<0\ 01$) and further to 41 0±8 4 by the end of 24 hours of CNP therapy

Respiratory frequency

Within 1 hour after CNP therapy was initiated respiratory rates started to decrease Although this trend toward improvement persisted through 48 hours respiratory rates did not become significantly different from the pre CNP mean value until the end of 24 hours ($p<0\ 01$) During the first 12 hours of therapy decreases in respiratory frequency were negatively correlated with increases in CNP ($p<0\ 005$)

Intrapulmonary right to left shunt

From a pre CNP mean of 28 7±3 8% intrapulmonary shunt decreased to 24 4±2 2% by the end of 24 hours of CNP therapy ($p<0\ 01$)

Mean values for blood pressure heart rate pH or $PaCO_2$ did not change significantly during the first 24–48 hours of CNP therapy However after initial improvement each of the four patients who died developed progressive hypoxemia, carbon dioxide retention increases in intrapulmonary right to-left shunt metabolic and respiratory acidosis tachycardia and hypotension a few hours before death

Complications

Four patients developed pneumothorax that was successfully decompressed in each case This complication occurred after 18 hours of therapy and only in those patients who had received more than 12 cm H O of negative pressure In two instances the pneumothorax involved a lung that had been the site for aspiration by a percutaneous transthoracic technique Moderate neck abrasions were noted in six patients Excessive cooling of the body did not occur None of the children showed undue apprehension while they were in the respirator nor did any survivors have adverse psychological reactions

Outcome of disease

Ten patients survived Autopsy results available for three of the four fatal cases included *diffuse and confluent alveopathy of varying severity* This process consisted of a reduction in alveolar space due to proliferation of hyper

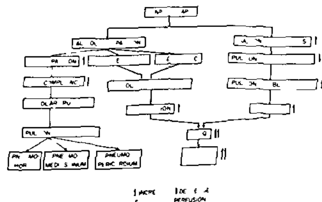


Fig 3 Proposed mechanism for effects of CNP therapy Solid lines (—) indicate the possible mechanisms for beneficial effects of CNP therapy whereas the broken lines ( ) indicate the adverse complications of pulmonary air leak VA/Q denotes ventilation perfusion ratio

plastic epithelial cells lining the alveoli and to proteinaceous eosinophilic exudate containing macrophages Widening of the intra alveolar septae due to fibrosis mononuclear cell infil trates and proliferation of congested capil laries were noted Lung specimens from two patients contained *Pneumocystis carinii* cysts

DISCUSSION

Progressively severe hypoxemia that is usually refractory to conventional modes of oxygen therapy is a common cause of death in im munosuppressed patients with diffuse bilateral alveolar disease (8 10) Management of such patients has previously required prolonged ex posure to hight ambient oxygen concentra tions with the attendant risk of irreversible pulmonary oxygen toxicity Results of the present study confirm our preliminary impres sions (17) that CNP therapy substantially im proves arterial oxygenation in spontaneously breathing older children who had severe hy poxemia of pulmonary origin Of added signi ficance such therapy eliminates endotracheal intubation and the use of muscle relaxants and sedatives and permits an early reduction in oxygen concentrations thus minimizing the hazards of oxygen toxicity

The high survival rate in this study 10 of 14 patients is in sharp contrast to our earlier ex perience with five other immunosuppressed children who presented with clinical and roentgenologic evidence of diffuse alveolar disease After detection of *P carinii* orga nisms in lung aspirates each patient was given pentamidine intravenous fluids and controlled oxygen therapy that was monitored on the basis of sequential blood gas analysis All five patients developed progressive respiratory in sufficiency and required an increase in FIO_2 Subsequently because of an inability to main tain $PaO_2 \geq 70$ mmHg despite an increase in FIO_2 to 60% or above ventilatory support with a pre set volume ventilator and PEEP was initiated The disease ended fatally in each child Similar experiences have been re ported by other investigators who appear to have met with little success using pre set vol ume respirators or membrane oxygenators in the management of progressive respiratory sufficiency due to *Pneumocystis carinii* pneu monitis (4 8 9 21)

The exact manner in which CNP therapy improves arterial oxygenation is not clear In some patients with lung disease closing capaci ty (CC) may exceed functional residual capac ity (FRC) during tidal breathing and thus pro- duce arterial hypoxemia That application of CNP around the chest wall can increase FRC has been demonstrated by Bancalari et al (2) Further Abboud et al and others (1) have shown that application of continuous distend ing airway pressure decreases closing volume Thus an increase of FRC in the face of a de crease in closing volume may reduce areas with airway closure and improve arterial oxy genation In Bancalari s study however the reported increases in FRC were not linearly related to improvements in arterial oxygena tion which suggests that the beneficial effects of CNP could be related to factors other than an improvement in the relationship between FRC and closing volume

These possible contributing factors include an increase in vascular transmural pressure (5) with a consequent decrease in pulmonary vascular resistance (16) as well as an increase in pulmonary blood flow (13) Increased pul monary blood flow in the presence of an in crease in thoracic gas volume would be ex pected to improve ventilation perfusion ratios and hence would explain the improvement in arterial oxygenation that was seen in most of our patients following the initiation of CNP therapy On the other hand a progressive in crease in negative pressure beyond a certain level can produce alveolar overdistention with a decrease in lung compliance (2) and subse quent rupture of the distended alveoli with development of pulmonary air leak as oc curred in four of our patients

Apparently then the effects of CNP ther apy depend upon whether the primary re

sponse involves recruitment of nonventilated alveoli or progressive overdistention of these structures (Fig 3) The former response would improve arterial oxygenation and thus benefit the patient whereas the latter after a certain degree of distention would compromise lung compliance and might even produce the complication of pulmonary air leak Recently Suter et al (20) reported an optimum pressure for producing beneficial effects in adults receiving positive end expiratory pressure (PEEP) for acute respiratory failure Whether there is a similar optimum level of CNP therapy for children needs further careful evaluation of cardiopulmonary hemodynamics and pulmonary function status before during and after this mode of therapy

ACKNOWLEDGEMENTS

We thank the members of the Intensive Care Unit Nursing Team and the Pediatric House Staff for care of these patients William C Maurencheck M D and Alvin M Mauer M D for their critical review of the manuscript and Mr John Gilbert for his help in preparing the manuscript

Supported by Cancer Research Center Grant CA-08480 and Training Grant CA-05176 from the National Cancer Institute and by ALSAC

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Submitted Aug 6 1976
Accepted Dec 30 1976

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Acta Pædiatr Scand 66 457–463 1977

CROSS SECTIONAL STUDIES OF PHYSICAL GROWTH IN TWINS POSTMATURE AND SMALL FOR DATES CHILDREN

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ABSTRACT Chamberlain R N and Simpson R N (British Births Child Study Paediatric Unit St Mary s Hospital Medical School London England) Cross-sectional studies of physical growth in twins postmature and small for-dates children Acta Paediatr Scand 66 457 1977.—From 16 955 live births which occurred throughout the United Kingdom in April 1970 three groups of children who might have suffered from fetal malnutrition i e the multiple postmature and small for-dates births were selected with a 10% random sample Their birthweights and weights heights and head circumference measurements were compared at the ages of 22 months and 3½ years Differences between the random and postmature groups still present at 22 months disappeared at 3½ years Variations in the other groups persisted and the compensation occurring at 22 months had apparently ceased at 3½ years The three examinations were linked and the children including those in the control group showed considerable mobility in their quartile ratings Only a minority remained unchanged At the same time the weights of the heavier and lighter children tended to go towards the mean As the velocity of growth slowed the redistribution tended to lessen but was still taking place at the age of 3½ years

KEY WORDS Twins postmature small for-dates fetal malnutrition physical growth

To determine whether fetal malnutrition might have a permanent effect on the subsequent physical and mental development of children three groups were selected for study In the first the twins it was assumed that growth retardation might have occurred from competition between the fetuses for the available nourishment In the second the postmature babies it was thought that cessation of placental growth about the 39th week of pregnancy (7) could have resulted in malnutrition in the month or so prior to birth The third consisted of the small for dates babies who were selected because their birthweights showed evidence of growth retardation at every gestational age None of these three groups is homogeneous but each is likely to contain substantial numbers of children who could have suffered from fetal malnutrition

PLAN OF INVESTIGATION

The children were selected from the national cohort of 16955 live births in the British Births Survey which included over 95% of the births which took place during the week beginning 5 April 1970 throughout the United Kingdom (1) From the information collected at birth the sample was drawn as follows

(i) A 10% random sample of all singleton and multiple births

(ii) All multiple births i e twins and triplets

(iii) All singleton children who were born at or later than 42 weeks gestation whose mothers were recorded as being *certain of the date of their last menstrual period* and not to have taken the contraceptive pill for at least 15 months before childbirth

(iv) All singleton children who were on or below the 5th centile of *birthweight for their gestational age allowing for* sex and maternal parity but regardless of whether their mother was certain of her last normal menstrual period or had taken the contraceptive pill These children were identified using *Tanner & Thomson s charts for birth* weight and length of gestation (8)

The children of mothers who stated that they were unmarried divorced separated or widowed at childbirth

were then excluded because of the difficulties involved in tracing them The random sample was compared with its British Births Survey equivalent and no statistical difference was found below the 5% level in any of the observed characteristics

The method of sampling tracing the children and the results of the second examination have already been published (2 3) so that only an outline is given here

The groups were not mutually exclusive and related to 3471 children of whom 96 had died all between birth and the second examination 2555 or 73 6% were seen between the ages of 92 and 123 weeks or were known to have died about half being in the age range of 94 to 97 weeks At the third examination 2426 or 69 9% were seen between the ages of 3 years 16 weeks and 4 years 8 weeks or were known to have died two-thirds being seen during the twelve weeks between 3 years 18 weeks and 3 years 29 weeks Some children untraced at the second examination were found at the next and vice versa Thus 2055 or 60 9% of the living children were examined at birth and on the two subsequent occasions

Chi squared and two sample t tests were used to test for sampling errors No significant difference was found between those examined between 3 years 18–29 weeks and those examined earlier later or not examined for sex maternal height and age in any of the groups However of those examined during the twelve week period the average birthweight was slightly heavier in the postmature group and the mean gestational age was marginally longer in the small for-dates group Although there was no difference in the distribution of Social Class I–V among those examined in the twelve week period there was a slightly smaller proportion of children with fathers from the Armed Forces in the postmature and random groups there was a slightly smaller proportion of children whose mothers were having their second or third baby in the small for-dates group there was a slightly higher proportion of mothers who came from Northern Ireland among the random and a slightly smaller proportion from the West Indies among the random and twin groups None of these differences was great and only affected proportions of numerically small groups so that they are unlikely to affect the results given here

Minor differences between the proportions of children examined in each of the three four week periods between the ages of 3 years 18–29 weeks were also found for the above variables but again are unlikely to affect the results given here

The analysis

The data has been analysed as three cross sectional studies and as a longitudinal study

(*a*) *Cross sectional studies* Two sample t tests were applied to compare the differences in the physical measurements of the three groups from the random sample for boys for girls and for both sexes together The analysis included only children where measurements had not presented difficulties e g *refusal to stand still* Differences found between the results at the second and third examinations were checked by using only those children examined both times The relation between height and weight was studied using an analysis of covariance

(*b*) *Longitudinal study* The patterns of growth were analysed by dividing the heights and the weights of the children at each examination into quartiles and comparing the differences For birthweight quartiles the standards published by Thomson et al (10) which take into account sex and gestational age up to 42 weeks for singleton children were used For the height and weight quartiles the standards published by Tanner et al (9) which take into account age and sex were used but as figures were given for length only under the age of 2 years heights were extrapolated using the data for 2 years of age where both length and height were given

The data in the longitudinal study refers to a slightly different group of children than that in the cross sectional studies for as the quartiles were standardized for age it was possible to include a wider sample although restricted to those examined on all three occasions

The control group now refers to singleton children with gestational ages of 38–41 weeks (inclusive) who were examined between the ages of 92–109 weeks in the second examination and 3 years 18–29 weeks in the third and where no difficulties were recorded in taking the measurements There was no statistical variation below the 5% level between the quartile distributions of birthweight weight and height for this control group and the standards

To make them compatible the standards for birth weights of singleton children were used for the twins with gestational ages of 38–41 weeks (inclusive)

For postmature children those with gestational ages of 42 and 43 weeks were included but the standards for birthweights of 42 weeks only were used as others were not available

For the small for-dates children those with gestational ages of 38–41 weeks were included The analysis was carried out on both sexes with similar findings but to avoid repetition the results of the longitudinal study are only given for boys

RESULTS

Random sample

Table 1 gives the mean physical measurements of the boys and the girls between the ages of 3 years 18–29 weeks in the third cross sectional study At 94–97 weeks in the second study (2) the boys were significantly heavier than the girls ($p<0.001$) and this variation has remained They were still taller but the degree of difference was not so great ($p<0.001$ in the second and $p<0.01$ in the third) The velocity of growth in height of girls is quicker than that of boys until about the age of 4 years when it is the same for both sexes (9) and this probably accounts for the differences found between the two cross sectional studies

Table 1 *Mean physical measurements of boys and girls aged 3 years 18 weeks to 3 years 29 weeks excluding those with difficulties in measurements*

Measurements	Random			Twins			Postmature			Small		
	No	Mean	S D	No	Mean	S D	No	Mean	S D	No	Mean	S D
Boys												
Age (wks over 3 yrs)	576	23 1	2 8	105	22 9	2 7	302	23 3	2 8	292	23 0	2 6
Weight (kg)	465	15 56	1 89	99	15 12	1 74	290	15 53	1 90	255	14 48	1 79
Height (cm)	478	98 0	4 4	98	96 8	4 4	301	98 1	4 6	263	95 6	4 2
Head size (cm)	478	51 3	1 6	96	51 0	1 6	300	51 2	1 6	267	50 3	1 6
Girls												
Age (wks over 3 yrs)	478	23 3	2 7	93	23 4	2 6	294	23 0	2 6	216	23 4	2 8
Weight (kg)	439	15 00	1 99	92	14 52	1 86	274	14 89	1 95	187	13 72	1 83
Height (cm)	449	97 3	4 3	88	96 7	5 1	278	96 9	4 7	200	94 2	5 1
Head size (cm)	437	50 1	1 5	92	49 9	1 5	274	49 9	1 7	197	49 2	1 6

Table 2 shows the number and proportion of boys in each of the quartiles at the three examinations and Fig 1a shows the proportion whose weights at 92–109 weeks were in the same quartiles as their birthweights Those whose birthweights lay in Quartile I i e on or *below the 25th centile* could only remain unchanged or move to a higher quartile those in Quartile IV could only remain unchanged or move to a lower quartile while those in the middle quartiles were able to remain unchanged or move either up or down If such changes had been uniform throughout the sample and unaffected by birthweight then it would be expected that the proportion remaining unchanged in each of the inner quartiles would be about one third compared with two thirds in each of the outer groups In fact 30% and 29% were unchanged in the two inner quartiles and 35% in the first and 39% in the fourth Thus although a larger proportion of the children in *the outer quartiles remained* unchanged there was actually a greater mobility than expected amongst these children

Table 2 *Number and proportion in each weight quartile at the three examinations (boys only)*

Quartiles	Random 263		Twins 36		Postmature 190		Small 152	
	No	%	No	%	No	%	No	%
At birth								
I	79	30 0	26	72 2	55	28 9	152	100 0
II	57	21 7	5	13 9	45	23 7	–	–
III	70	26 6	5	13 9	40	21 1	–	–
IV	5	21 7	0	0 0	50	26 3	–	–
At 92–109 weeks								
I	59	22 4	10	27 8	48	25 3	74	48 7
II	68	25 9	7	19 4	53	27 9	41	27 0
III	65	24 7	13	36 1	49	25 8	26	17 1
IV	71	27 0	6	16 7	40	21 1	11	7 2
At 3 years 18–29 weeks								
I	70	26 6	12	33 3	48	25 3	79	52 0
II	59	22 4	11	30 6	49	25 8	39	25 6
III	64	24 3	11	30 6	42	22 1	20	13 2
IV	70	26 6	2	5 5	51	26 8	14	9 2

Singleton boys with gestational ages of 38–41 weeks (inclusive)
Boy twins with gestational ages of 38–41 weeks (inclusive)
Singleton boys with gestational ages of 42 and 43 weeks

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III	70	6 6	5	13 9	40	21 1	–	–
IV	57	1 7	0	0 0	50	26 3	–	–
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III	65	24 7	13	36 1	49	25 8	26	17 1
IV	71	27 0	6	16 7	40	21 1	11	7 2
At 3 years 18–29 weeks								
I	70	26 6	12	33 3	48	25 3	79	52 0
II	59	22 4	11	30 6	49	25 8	39	25 6
III	64	24 3	11	30 6	42	22 1	20	13 2
IV	70	26 6	2	5 5	51	26 8	14	9 2

Singleton boys with gestational ages of 38–41 weeks (inclusive)
Boy twins with gestational ages of 38–41 weeks (inclusive)
Singleton boys with gestational ages of 4[illegible] and 43 weeks

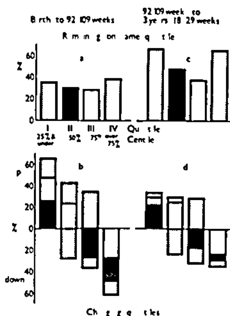


Fig 1 Quartile distributions of weights of singleton boys with gestational ages of 38–41 weeks in the control sample

Fig 1*b* shows that from each quartile the weights of the children were redistributed about the mean for the whole cohort and the degree of redistribution was in much the same order for each quartile

Figs 1*c* and *d* illustrate the changes between 92–109 weeks and 3 years 18–29 weeks 47% and 39% of the children in the two inner quartiles remained unchanged If the movements for the outer quartiles had been of the same order it would be expected that the position of about 70% would have remained unaltered In fact 66% of those in Quartile I and 65% of those in Quartile IV were unchanged Thus again in each quartile the weights were redistributed around the mean with a slightly greater tendency for the lighter and heavier children to alter their position but not to the same extent as in the earlier examination When the results of the three cross sectional studies were considered together the weights of only 19 4% of the boys remained consistently in the same quartile while 17 1% had changed at each examination including 4 6% who showed a steady increase and 2 7% who showed a steady decrease

Length was not recorded at birth so that figures for height are only for the two later examinations (Table 3) when the patterns were similar to those of the weights

Twins

198 or 64 1% of the 309 twins were examined between the ages 3 years 18–29 weeks They were lighter and shorter ($p<0\ 01$) but their body build did not vary significantly from that of the random group so there was no evidence that they had caught up and if anything the gap had widened This was confirmed by repeating the analysis using only those children who had been examined both times Their mean head size was significantly different from that of the random group ($p<0\ 05$) so that on average they had relatively large heads for their bodies

Table 2 shows the quartile distribution of the weights of the 36 male twins The number of fullterm twins is small so figures have not been included as they might have been misleading At birth the weights of almost three quarters were in the first while none were in the fourth quartile By 92–109 weeks considerable movement had taken place and six children had reached the fourth quartile three

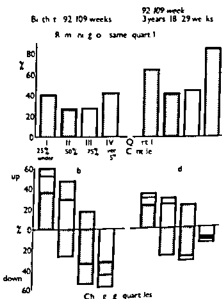


Fig 2 Quartile distributions of weights of singleton boys with gestational ages of 42 and 43 weeks in the post mature group

Table 3 *Number and proportion in each height quartile at the three examinations (boys only)*

Quartiles	Random 284		Twins* 34		Postmature 212		Small * 16?	
	No	%	No	%	No	%	No	%
At 9?–109 weeks								
I	78	27 5	9	26 5	6?	29 2	77	47 5
II	67	23 6	10	29 4	45	?1 2	38	?3 5
III	?0	?4 6	10	?9 4	55	?5 9	35	?1 6
IV	69	24 3	5	14 7	?0	23 6	12	7 4
At 3 years 18–29 weeks								
I	6?	21 8	6	17 6	46	21 7	7?	44 4
II	63	2? 2	6	17 6	49	23 1	3?	20 4
III	81	28 5	14	41 2	53	25 0	44	27 2
IV	78	27 5	8	23 5	64	30 2	13	8 0

Singleton boys with gestational ages of 38–41 weeks (inclusive)
* Boy twins with gestational ages of 38–41 weeks (inclusive)
Singleton boys with gestational ages of 42 and 43 weeks

of whom had birthweights in the first Between 92–109 weeks and 3 years 18–29 weeks the re distribution was similar to that of the control group in Quartiles I and II but in III and IV only a few children remained unchanged and with one exception those who moved went downwards Ten (28%) of the 36 twins re mained in the same quartile at each examina tion and 12 (33%) were in different ones in cluding 3 (8%) with a steady increase and 1 with a steady decrease

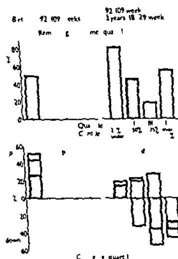


Fig 3 Quartile distributions of weights of singleton boys with gestational ages of 38–41 weeks in the small for dates group (i e on or below the 5th centile)

The distribution and mobility of heights be tween 92–109 weeks and 3 years 18–29 weeks were similar to those of the weights except that at the third examination there was a higher proportion of children in the upper quartiles for height compared with weight (Table 3) An analysis of covariance confirmed that compared with the control group the twins were light for their height

Postmature

Of the 972 postmature children 616 or 63 4% were seen at the third examination Their measurements showed no significant differ ence from those of the random group and whereas they had appeared light for their height at 94–97 weeks they now appeared to be of the same build

Table 2 shows the proportions of the weights of the boys in each of the four quar tiles at the three examinations and Fig 2 il lustrates their distribution and mobility The results were similar to those of the control sample although in the third quartile at 92–109 weeks a smaller proportion moved into the first quartile and a higher proportion into the second compared with the control group The weights of 22 6% of the postmature boys were in the same while 16 8% were in different quartiles at each of the three examinations in

cluding 6 3% who moved up and 4 7% who moved down consistently

Study of the height quartiles gave similar results to those of weight (Table 3)

Small for dates

Of the 799 children 508 or 63 6% were examined These children remained lighter and shorter with smaller head circumference measurements than the random group ($p<0\ 001$) An analysis of covariance showed that individually the children were light for their heights ($p<0\ 001$)

Although all the birthweights of the small for dates boys were in the lower fifth of the first quartile (Fig 3*a*), by the second examination less than half had remained in the first and 7 2% had reached the fourth quartile (Fig 3*b*) At the third examination 81 1% of the boys had remained in the first quartile and whereas between the first two examinations the weight of about half the children had moved upwards between the last two the tendency was to move down rather than up (Figs 3*c* and *d*) Compared with 19 4% of the control sample, 39 5% of the group had not changed quartiles throughout

The distributions of weights and of heights within the quartiles were not significantly different at the second examination At the third, 52% were in the first quartile for weight while the equivalent for height was 44% so that overall the boys appeared to be light for their height In Quartiles I, III and IV there was no significant difference in the proportions who stayed or changed to other quartiles whereas in Quartile II the heights were redistributed to higher quartiles than the weights

DISCUSSION

The results from the two cross sectional studies at birth and at 94–97 weeks of age showed that at the second examination the pattern of distribution of weights was much closer to the normal than that of the birthweights but that differences still remained between the groups selected and the random group (2)

By the age of 3 years 18–29 weeks the differences on average, between the postmature and random groups had disappeared so that if postmaturity had been the cause of fetal malnutrition there is nothing to suggest that it had had a permanent effect on the subsequent physical progress of the children On the other hand, the variations in each of the other groups had persisted and were of the same order as those found at 94–97 weeks Thus although there was considerable evidence of compensation at the second examination this had apparently ceased by the third

By linking the three examinations together the changes could be considered in greater detail Falkner (5) has suggested that once a healthy infant has shaken off the perinatal factors influencing early growth he climbs on to a largely genetically determined series of individual trajectories and then continues along them He considers it takes about 2 years to get 'on target While the above results are generally in agreement with this when the movements from one quartile to another are considered, only a small proportion of the children remained on target from birth to about 3½ years and only about half from about 22 months to 3½ years Thus from birth there was considerable variation in the growth rates of all children with, as might be expected a greater tendency for the weights of the heavier and lighter children to redistribute around the mean As the velocity of growth slowed down the extent of this redistribution tended to lessen but was still taking place at the age of about 3½ years

These findings have strengthened the suggestion in the earlier report (2) that centile charts should be used with caution when monitoring the growth of individual children Large or small children although overall exhibiting hold back or catch up growth are more likely to remain in the same quartiles than those in the middle quartiles and it would seem normal for many children to cross centile lines If

centile charts are used for dietary control (4 6) we would suggest that they should only be used as part of the general assessment of the health of the child taking into account the birthweight and gestational age as well as the height or length It would seem unwise to rely on one or two measurements only

ACKNOWLEDGEMENTS

We wish to thank Professor T E Oppé of St Mary s Hospital Medical School and Professor B Benjamin of City University for their assistance the Medical Research Council for providing the funds the National Birthday Trust Fund for use of the British Births Survey data the doctors who undertook the examinations and the health visitors and administrative staff who traced and found the children

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Submitted July 20 1976
Accepted Jan 20 1977

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Acta Pædiatr Scand 66 465–471 1977

ATOPIC DISEASE IN SEVEN YEAR-OLD CHILDREN

Incidence in Relation to Family History

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From the Department of Paediatrics Linkoping University Linkoping Sweden

ABSTRACT Kjellman N I M (Department of Paediatrics Linkoping University Sweden) Atopic disease in seven year-old children Acta Paediatr Scand 66 465 1977 —The incidence of atopic disease and its relation to the family history was studied by questionnaire in 1325 children 7 years of age A higher incidence of bronchial asthma (2 7%) was found than in a previous Swedish study The total incidence of atopic disease in the children was 15 1% with a higher level when there was a double parental history of such disease (42 9%) as compared with a single such history (19 8%) When both parents had an identical type of atopic disease i e respiratory or skin the incidence of atopic disease was higher (72.2%) than when non identical types occurred in the parents (20.8%) The findings support theories of a polygenic transmission of atopic disease as well as a genetic influence on symptom specificity in such disease and may be of value in genetic counselling

Atopic dermatitis bronchial asthma and allergic rhinitis as well as certain forms of gastrointestinal allergy and urticaria are usually referred to as atopic diseases (18) These diseases show a familial clustering as early as 1650 a familial occurrence of bronchial asthma was reported by Sennertus (23) However there is no agreement about the mode of genetic transmission of the tendency to atopy (2 20 23) The time of onset and the intensity of atopic disease are also decided by genetic (20) and environmental (18) factors

The total incidence of atopic disease in European and North American children is reported to be 12–24% (1 7 16) The incidence of childhood asthma is reported to have increased in the US and the UK in recent decades (15 17) No current data are available on the incidence of atopic disease in Sweden

Children with atopic allergy are more susceptible to infections than others (3) and they also have more respiratory infections and more episodes of otitis media than non atopic children (5)

The present study was initiated by an interest in the actual incidence of atopic disease in Swedish children as well as in the genetic transmission of such disease It seemed of further interest to find out if in fact there is any genetic linkage between a tendency to atopy and recurrent episodes of otitis media

MATERIAL AND METHODS

All 1473 school beginners (7 years of age) in the community of Linköping Sweden were interviewed during 1975 by means of quesionnaire The investigation was coordinated with the usual health survey of the children The questionnaire concerned individual as well as family histories of atopic disease (atopic dermatitis allergic rhinitis allergic urticaria and bronchial asthma) and of recurrent otitis media (defined as otitis media four times or more) The families were also asked to give a short description of possible allergic symptoms in the family members (the school beginner as well as his/her parents

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Table 3 *Individual histories of the children in relation to sex*
For abbreviations see Table 1

Probands		Total	BA	AR	AD	U	Atopic disease (one or more)	ROM
Boys	n	678	27	40	49	17	114	194
	%	51 2	3 9	5 9	7 2	2 5	16 8	28 6
Girls	n	647	9	10	61	10	86	177
	%	48 8	1.4	1.5	9 4	1.5	13 3	27 4
	p		<0 01	<0 001	n s	n s	n s	n s
Total	n	1 325	36	50	110	27	200	371
	%	100 0	2 7	3 8	8 3	2.0	15 1	28 0

tory of atopic disease (Table 2) the incidence of such disease was higher (42 9%) than in the children with a single such heredity (19 8%) in whom however the incidence was higher than in children without any parental history of atopic disease (12 5%) Significantly higher incidences of the 4 studied atopic diseases were also found in children with a double parental history of atopic disease (Table 2) the following p values were found

bronchial asthma	<0 01
allergic rhinitis	<0 01
atopic dermatitis	<0 001
urticaria	<0 05

Comparison of children with a single parental history of atopic disease with those without such history revealed that only atopic dermatitis had occurred more often in the former ($p<0$ 001)

Two or more atopic diseases in the same child occurred more often in those with a double parental history of atopic disease than in any of the other groups (Table 2)

No significant difference was found in the atopy incidence whether a sibling and a parent or only a sibling had atopic disease (*Fig* 2) Rather atopic disease occurred more often with an atopic sibling *than with one* atopic parent ($p<0$ 01)

The incidence of atopic disease in the children was significantly higher ($p<0$ 001) if both parents had an *identical type* of atopic disease i e respiratory or skin (Table 4) than when non identical types occurred in the parents The incidence of atopic disease *in the children* in whom only one parent had a history of atopic disease was significantly higher when the parent had a *combined* type of atopic disease than when there was only one type of such disease in the parent ($p<0$ 01)

Recurrent otitis media was reported to have occurred in 6 5% of the *parents* (*Table 1*) A double parental history of such disease was *found* in 24 (1 8%) of the children (Table 5) and a single such history in 123 children (9 3%) A further 219 children (16 5%) without a parental history of such *disease had* a sibling with recurrent otitis media Thus in all *366 children (27 6%)* had a family history of recurrent otitis media

The incidence of recurrent otitis media in

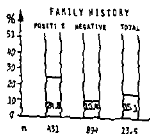


Fig 1 Incidence of atopic disease in children with a positive and negative family history of such disease n= no of *children with the family history* in question

Table 1 *Parental histories*

BA=bronchial asthma AR=allergic rhinitis AD=atopic dermatitis U=allergic urticaria ROM=recurrent otitis media

Probands		Total	BA	AR	AD	U	Atopic disease (one or more)	ROM
Fathers	n	1 312	26	76	68	21	176	78
	%	100 0	2 0	5 8	5 2	1 6	13 4	5 9
Mothers	n	1 325	25	77	126	27	201	93
	%	100 0	1 9	5 8	9 5	2 0	15 2	7 0
Total	n	2 637	51	153	194	48	377	171
	%	100 0	1 9	5 8	7 4	1 8	14 3	6 5

and siblings) These descriptions were used in the classification of the diseases (atopic or non-atopic)

Answers were received concerning 1325 children (=90 0% of all school beginners) The χ^2 test was used to evaluate group differences

RESULTS

Atopic disease was reported to have occurred in 14 3% of the parents of the 1325 seven year-old children (Table 1) A double parental history of atopic disease was found in 42 of the children (3 2%) and a single such history in 293 (22 1%) of them (Table 2)

In a further 96 children (7 2%)—without parental history of atopic disease—a sibling was reported to have suffered from such disease In all, 431 of the group (32 5%) had a family history of atopic disease

The individual histories of the children in relation to sex are shown in Table 3 Allergic rhinitis and bronchial asthma were reported to have occurred significantly more often in boys than in girls The total incidence of atopic disease was 15 1% with no significant differences between boys and girls The incidence was 24 8% in those with a family history of atopic disease and 10 4% in those without (Fig 1) A family history of atopic disease was reported in 49 5% of the children with such disease The incidence of a positive family history of atopic disease was 73 5% in children with two or more different types of atopic diseases in contrast to 44 6% in children who had only one manifestation of atopic disease ($p<0\ 01$)

In the children with a double parental his

Table 2 *Incidence of a history of atopic disease in parents and children*

For abbreviations see Table 1

Parental history of atopic disease		Total	Atopic disease in the children							
			BA	AR	AD	U	One or more	p	Two or more	p
Double	n	42	6	7	14	4	18		6	
	%	3 2	14 3	16 7	33 3	9 5	42 9	<0 001	14 3	<0 001
Single	n	293	9	14	36	7	58		5	
	%	22 1	3 1	4 8	12 3	2 4	19 8	<0 001	1 7	<0 01
None	n	990	21	29	60	16	124		2	
	%	74 7	2 1	2 9	6 1	1 6	12 5		0 2	
Total	n	1 325	36	50	110	27	200		13	
	%	100 0	2 7	3 8	8 3	2 0	15 1		1 0	

Table 5 *Incidence of recurrent otitis media (ROM) in unselected school children in relation to the parental history of and atopic disease (= ATOPY)*

Parental history	n	%	ROM in the children n	%	p
ROM					
Double	24	1.8	19	79 ?	<0 01
Single	1?3	9 3	?8	47 2	<0 001
None	1 178	88.9	294	25 0	
Total	1 325	100 0	371	28 0	
Atopy					
Present	332	25 1	109	32 8	<0 05
Not present	993	74 9	26?	26 4	
Total	1 325	100 0	371	28 0	

tulsky (2) among others In addition the incidence of a positive family history of atopic disease in the children with atopic disease (49 5%) is in agreement with previous findings (2)

The incidence of a history of bronchial asthma in the children (2 7%) was higher than in a previous Swedish study (1 4% 12) A similar increase has been reported in UK children in recent decades (15) and an even higher incidence (6 3%) of bronchial asthma was found in Australian children (22) Genetic and environmental as well as diagnostic differences may contribute to such discrepancies

The total incidence of atopic disease (15 1%) was similar to incidences reported by Halpern et al (7) in 1973 (12 4% in children from 0 through 7 years of age) and by Rapaport et al (16) in 1960 (19 4% during childhood) In a randomly selected sample of 164 eight year old children (9) 18 3% had a history of atopic disease

Atopic dermatitis is usually the first sign of atopy in childhood—most such cases have their onset in infancy A diagnosis of bronchial asthma is more often made in children 1–6 years of age whilst allergic rhinitis symptoms usually begin between 5 and 19 years of age Hence the total incidence of atopic disease is influenced by the age of the children who are studied It is thus not surprising that the most common disease in the 7 year-old children was atopic dermatitis The higher incidence of allergic rhinitis and bronchial asthma in boys (Table 3) is in accordance with the findings of others (1)

The presence of parental atopic disease was found to be of great importance for the incidence of such disease in the offspring This influence was obvious with regard to the total incidence the type and the intensity of atopic disease in the children

Children with a double parental history of atopy had developed atopic disease twice as often as those with a single such parental history The differences in incidence when both one or no parent was affected indicate a polygenic transmission Similar incidence figures were presented by van Arsdel & Motulsky (2) who also suggested that more than one gene locus must be involved Others have found support for a dominant (20) or recessive autosomal transmission (23) Twin studies (4) do not support any particular type of genetic transmission

In the 18 families in whom the parents had an identical type of atopic disease (i e respiratory or skin type) the toal incidence of atopic disease in the children was high (72 2% Table 4) In all of these families the same disease was found in the parents and the offspring Although this group was small it supports the evidence of a genetic influence on symptom specificity (4 23) The findings may be explained by a genetically determined end organ sensitivity as was recently described by Gerrard et al (6) Turner et al (22) found an additive influence of a biparental disease only in allergic rhinitis but not in bronchial asthma whereas this study favours such an additive influence in both of these repiratory allergies as well as in atopic dermatitis and allergic urticaria

The intensity of atopic disease as measured by the number of symptoms expressed in the

Table 4 *Comparison between parents and children with regard to the types of atopic disease*
Respiratory=BA and/or AR Skin=AD and/or U

Parental history	n	Atopic disease in the children: Respiratory	Skin	Combined	Total n	Total %	p
Double							
Respiratory	6	3	0	1	4		
Skin	12	0	8	1	9		
Identical	18	3	8	2	13	72 2	<0 001
Non identical	24	0	2	3	5	20 8	
Single							
Respiratory	128	4	14	1	19	14 8	
Skin	133	11	15	1	27	20 3	
Isolated	261	15	29	2	46	17 6	<0 01
Combined	32	1	9	2	12	37 5	

the *children* was 28 0% (Table 5) with the highest incidence in children with a double parental history of such disease (79 2%) and the lowest in those without such a parental history (25 0%)

The incidence of recurrent otitis media in the children was found to be higher ($p<0.05$) in children with a positive parental history of *atopic* disease than in those without However the incidence of recurrent otitis media was similar in the children with (64/200) and without (307/1 125) atopic disease

The duration of breast feeding did not seem to influence significantly the incidence of atopic disease nor the tendency to recurrent otitis media

DISCUSSION

The difficulty of establishing a diagnosis is well known in allergy research (8) The current figures for the incidence of atopic disease which are based on questionnaire data are of course subject to response errors by the parents Even when a child is submitted to a thorough examination and laboratory investigations it may be impossible to categorize the child as allergic or non allergic (11) Probably no such distinct categories exist—rather degrees of atopicity (8) The incidences of atopic disease reported to have occurred in the parents are probably somewhat too low as episodes of atopic dermatitis in infancy (for instance) are misunderstood or even forgotten However nearly identical incidences were reported to have occurred in the parents of the present study and those of 1827 newborns in the same community (9) bronchial asthma 1 9% allergic rhinitis 6 0% atopic dermatitis 7 6% and urticaria 1 7% This similarity suggests that the questionnaire data are valid

The total incidence of a positive family history of atopic disease (32 5%) is supported by the previous findings of van Arsdel & Mo

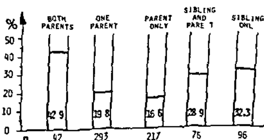


Fig 2 Incidence of atopic disease in children in relation to their family history of such disease *n*=no of children with the family history in question

Table 5 *Incidence of recurrent otitis media (ROM) in unselected school children in relation to the parental history of and atopic disease (= ATOPY)*

Parental history	n	%	ROM in the children n	%	p
ROM					
Doubl	4	1.8	19	79 2	<0 01
Single	173	9.3	58	47 2	<0 001
None	1 178	88 9	294	25 0	
Total	1 375	100 0	371	78 0	
Atopy					
Present	332	25 1	109	37 8	<0 05
Not present	993	74 9	767	26 4	
Total	1 325	100 0	371	8 0	

tulsky (2) among others In addition the incidence of a positive family history of atopic disease in the children with atopic disease (49 5%) is in agreement with previous findings (2)

The incidence of a history of bronchial asthma in the children (2 7%) was higher than in a previous Swedish study (1 4% 12) A similar increase has been reported in UK children in recent decades (15) and an even higher incidence (6 3%) of bronchial asthma was found in Australian children (22) Genetic and environmental as well as diagnostic differences may contribute to such discrepancies

The total incidence of atopic disease (15 1%) was similar to incidences reported by Halpern et al (7) in 1973 (12 4% in children from 0 through 7 years of age) and by Rapaport et al (16) in 1960 (19 4% during childhood) In a randomly selected sample of 164 eight year old children (9) 18 3% had a history of atopic disease

Atopic dermatitis is usually the first sign of atopy in childhood—most such cases have their onset in infancy A diagnosis of bronchial asthma is more often made in children 1–6 years of age whilst allergic rhinitis symptoms usually begin between 5 and 19 years of age Hence the total incidence of atopic disease is influenced by the age of the children who are studied It is thus not surprising that the most common disease in the 7 year old children was atopic dermatitis The higher incidence of allergic rhinitis and bronchial asthma in boys (Table 3) is in accordance with the findings of others (1)

The presence of parental atopic disease was found to be of great importance for the incidence of such disease in the offspring This influence was obvious with regard to the total incidence the type and the intensity of atopic disease in the children

Children with a double parental history of atopy had developed atopic disease twice as often as those with a single such parental history The differences in incidence when both one or no parent was affected indicate a polygenic transmission Similar incidence figures were presented by van Arsdel & Motulsky (2) who also suggested that more than one gene locus must be involved Others have found support for a dominant (20) or recessive autosomal transmission (23) Twin studies (4) do not support any particular type of genetic transmission

In the 18 families in whom the parents had an identical type of atopic disease (i e respiratory or skin type) the toal incidence of atopic disease in the children was high (72 2% Table 4) In all of these families the same disease was found in the parents and the offspring Although this group was small it supports the *evidence of a genetic influence on symptom specificity* (4 23) The findings may be explained by a genetically determined end organ sensitivity as was recently described by Gerrard et al (6) Turner et al (22) found an additive influence of a biparental disease only in allergic rhinitis but not in bronchial asthma whereas this study favours such an additive influence in both of these repiratory allergies as well as in atopic dermatitis and allergic urticaria

The intensity of atopic disease as measured by the number of symptoms expressed in the

Table 4 *Comparison between parents and children with regard to the types of atopic disease*
Respiratory=BA and/or AR Skin=AD and/or U

Parental history	n	Atopic disease in the children: Respiratory	Skin	Combined	Total: n	%	p
Double							
Respiratory	6	3	0	1	4		
Skin	12	0	8	1	9		
Identical	18	3	8	2	13	72 2	<0 001
Non identical	24	0	2	3	5	20 8	
Single							
Respiratory	128	4	14	1	19	14 8	
Skin	133	11	15	1	27	20 3	
Isolated	261	15	29	2	46	17 6	<0 01
Combined	32	1	9	2	12	37 5	

the *children* was 28 0% (Table 5) with the highest incidence in children with a double parental history of such disease (79 2%) and the lowest in those without such a parental *history (25 0%)*

The incidence of recurrent otitis media in the children was found to be higher ($p<0\ 05$) in children with a positive parental history of *atopic* disease than in those without However, the incidence of recurrent otitis media was similar in the children with (64/200) and without *(307/1 125)* atopic disease

The duration of breast feeding did not seem to influence significantly the incidence of atopic disease nor the tendency to recurrent otitis media

DISCUSSION

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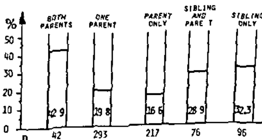


Fig 2 *Incidence of atopic disease in children in relation* to their family history of such disease *n*=no of children with the family history in question

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Submitted Oct 4 1976
Accepted Nov 25 1976

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parents, was also found to influence the incidence of atopic disease in the children in a child with a single parental history of atopic disease the incidence of such disease was significantly higher if the parent had a combined (i e consisting of more than one type of) rather than an isolated atopic disease Also in this respect the twin-data by Edfors Lubs (4) support the present findings

It seems probable that there is a genetic linkage between atopic disease and a tendency to recurrent otitis media as a family history of atopic disease was significantly overrepresented in children with recurrent otitis media Atopic children have more respiratory infections than their siblings or control groups (5, 14) Furthermore atopic individuals have an increased tissue reactivity to vasoactive amines in comparison with others (19) Thus immune responses are more violent in atopic children than in others The infectious swelling of the mucosal membrane is to some extent responsible for the 'asthmatoid character of bronchitis in young children In the upper respiratory tract the Eustachian tube is easily blocked by mucosal swelling and/or cellular infiltrations, and such obstruction predisposes to bacterial infection of the middle ear (13) In accordance with this a high incidence of atopic stigmata has been observed in children with recurrent otitis media and/or recurrent otosalpingitis as well as in children with nasal obstruction and/or repeated respiratory infections (10) Why some atopic children are more apt to nasal or bronchial conditions and others to otological problems may possibly be explained by their different terrain factors which are under a complex genetic influence (21)

In conclusion the incidence of atopic disease was high even before eight years of age in children of two atopic parents and especially high when the same type of allergy occurred in the parents Hence attempts should be made to increase the information given to atopic families (not forgetting the older children) as to the hereditary concept of atopic disease Such information may contribute together with an IgE screening in high risk newborns and an early recognition of a possibly atopic etiology in childhood disease to arrest further increase of atopic disease in childhood

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ON THE SOURCE OF LIPASE ACTIVITY IN GASTRIC CONTENTS

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ABSTRACT Bläckberg L Hernell O Fredrikzon B and Åkerblom H K (Department of Chemistry Section on Physiological Chemistry Department of Paediatrics University of Umeå Umeå Sweden and Department of Paediatrics University of Oulu Oulu Finland) On the source of lipase activity in gastric contents Acta Paediatr Scand 66 473 1977 —A convenient assay procedure for determination of the activity of pharyngeal lipase (gastric content lipase) using a long chain triglyceride as substrate is described Lipase activity in extracts of rat tongue salivas collected from the upper esophageal pouch from two human newborns with congenital esophageal atresia and in gastric content obtained from an infant with pyloric stenosis were studied Optimal lipase activities of the three enzyme sources were found in the same pH range During hydrolysis the composition of the products formed were also similar The data presented indicate that at least some of the lipase activity which is responsible for lipolysis in the stomach of the newborn originates in pregastric tissues

KEY WORDS Pharyngeal lipase gastric lipolysis esophageal atresia lipid digestion

A few years ago it was shown by Helander & Olivecrona (12) that digestion and absorption of lipids take place in the stomach of the suckling rat This aroused our interest for the possible physiological role of the gastric lipid digestion and also the source and the properties of the lipases involved Lipolytic activity has previously been found in gastric contents from several species i e the rat (4) the dog (8 14) and man (1 6 19) In healthy adult humans the lipase activities were comparatively low and gastric lipolysis was considered to be of minor importance under normal conditions (6) However in a recent study we have found that lipase activity is already present in gastric contents collected at birth and that this lipase activity has properties different from pancreatic lipase (9) The lipase activity in gastric contents was low in the fasting state but increased considerably during a test meal of pasteurized human milk (9) During such a test meal an appreciable amount of the human milk triglycerides are hydrolyzed to mainly diglycerides and fatty acids in the stomach of infants (9 15 16) This suggests that gastric lipolysis might be a physiologically important lipolytic mechanism in the newborn (15 16)

The origin of the lipase responsible for gastric lipolysis is the subject of much controversy Hamosh & Scow (11) recently showed that in the rat serous glands (von Ebner s glands) of the tongue contain a lipase that hydrolyzes triglycerides mainly to diglycerides and fatty acids They suggested that this lipase is responsible for gastric lipolysis in the rat A pregastric esterase which can hydrolyze triglycerides is also present in the pharyngeal tissues of calves (18) In man the source of the gastric lipolytic activity has not been established although a gastric mucosal origin has been suggested (6 19) However recently Ha

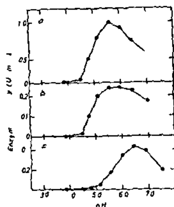


Fig 3 Effect of pH on the lipase activity The incubation system was prepared as described under Methods a 10 µl of enzyme solution (diluted 3 fold) prepared from rat tongue b 10 µl of esophageal aspirate from a human new born with congenital esophageal atresia c 10 µl of gastric contents from an infant with pyloric stenosis

individual tubes The pH value in each tube was recorded after the incubation

One lipase unit is defined as the amount of enzyme that releases 1 µmole of fatty acid per min at 37°C U×ml refers to lipase activity in 1 ml of rat tongue extract esophageal aspirate or gastric content

Determination of the reaction products Incubation mixtures were prepared as above except that tri(9 10(n)-H)-oleylglycerol was substituted for trioleyl(2(n ^{3}H) glycerol (The Radiochemical Centre Amersham England) 700 µl of the incubation mixture were incubated with varying amounts of rat tongue extract esophageal aspirate or gastric content in a total volume of 250 µl Incubation time was 15 min Extraction of lipids and separation of glycerides have been described previously (13)

RESULTS

The use of a radioactive labelled substrate permits accurate determination of very low lipase activities Optimal assay conditions were worked out using a rat tongue extract as enzyme source The amount of fatty acid released was linear with time (Fig 1) and enzyme concentration (Fig 2) until about 100 nmoles had been released into the medium This corresponds to a 10% hydrolysis of the triglycerides Under the conditions used optimal activity of the rat pharyngeal lipase was found in the pH interval 5 0–6 0 (Fig 3*a*)

To study the composition of the products formed during various degrees of hydrolysis different amounts of enzyme were added to the incubation system (Fig 4) For the rat enzyme (Fig 4*a*) there was a rapid release of diglyceride and a much slower release of monoglyceride When the largest amount of enzyme was used diglyceride was still the major partial glyceride found

Identical assay conditions were applied when lipase activity of esophageal aspirates obtained from the two newborns with esophageal atresia and of gastric contents collected from the infant with pyloric stenosis were determined Optimal lipase activity for esophageal aspirate was found in the pH range 5 5–6 5 (Fig 3*b*) and for gastric contents in the interval 6 0–7 0 (Fig 3*c*)

Lipase activities of the esophageal aspirates were 0 5 and 1 3 U×ml^{-1} respectively These activities are of the same magnitude as those found by Hamosh et al in human adults (10) and are in agreement with our previous observation that the ability to synthesize and secrete the pharyngeal lipase is already well developed in the newborn (9) The composition of the products formed during hydrolysis of emulsified triglyceride when either esophageal aspirate or gastric content were as

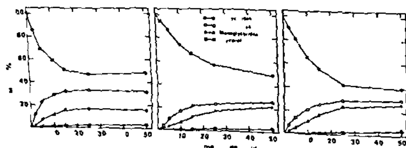


Fig 4 Composition of reaction products formed during hydrolysis of emulsified trioleate The incubations were performed as described under Methods Enzyme sources were varying amounts of (*a*) extract from rat tongue (*b*) esophageal aspirate (*c*) gastric contents

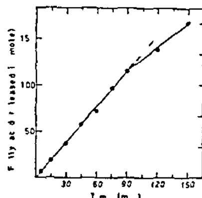


Fig. 1. Time course of the release of fatty acids during incubation with rat pharyngeal lipase. The enzyme solution (prepared as described under Methods) was diluted 6-fold. 10 µl was incubated according to the routine assay procedure.

mosh et al. (10) found lipase activity in esophageal aspirates from healthy adult humans suggesting a pregastric origin also for the human lipase. These authors suggested the name pharyngeal lipase which will be used in the present study.

The aims of the present study were: firstly to develop a convenient procedure for the determination of pharyngeal lipase activity using a long chain triglyceride as the substrate, secondly to find out if the gastric lipolytic activity in the human newborn originates from pregastric tissues and if so, thirdly to compare some properties of the human and the rat pharyngeal lipases.

METHODS

Preparation of enzyme sources. Sprague Dawley rats were killed under light ether anesthesia and the rear part of the tongues including the von Ebner glands were cut off and kept frozen until use. This part of the tongue is rich in lipolytic activity (11, 16). In each preparation 3–4 tongues were used. The tongues were minced with scissors and homogenized in 10 volumes of 0.01 M Tris HCl buffer, pH 8.5, by the use of a Potter Elvehjelm homogenizer. Homogenates were centrifuged for 30 min at 39000 g. The pellets were rehomogenized in 10 volumes of 0.01 M Tris HCl in 1.0 M NaCl, pH 8.5, and then centrifuged as described above. The second supernatant was then used as source of rat pharyngeal lipase.

Two fullterm infants with congenital esophageal atresia were investigated as follows: before operative treatment aspirates of esophageal fluid were collected from the upper esophageal pouch. The aspirates were immediately frozen and stored at −20°C until analyzed. On operation a broncho-esophageal fistula was found in one infant and a tracheo-esophageal fistula in the other. The fluid obtained from the first infant was watery and alcaline, pH 9.0. Where not otherwise stated the aspirate obtained from this infant has been used as the enzyme source in the experiments performed.

Gastric contents were collected from a 25 days old boy suffering from pyloric stenosis. The diagnosis was confirmed on operation. The pH of the sample was 3.6. The sample was centrifuged for 15 min at 12000 g. The clear phase below the upper fatty layer was used as enzyme source.

Assay systems. 2×10^7 cpm of purified (2) tri(9,10(n)-^{3}H)-oleylglycerol (The Radiochemical Centre, Amersham, England) was mixed with 25 mg purified (20) olive oil, 1.0 ml 10% gum arabic, 3.0 ml 0.2 M sodium citrate buffer, pH 6.0, and 2.0 ml distilled water were added. The mixture was cooled with ice-water and sonicated for 4 min at maximal effect in a 100 W-disintegrator (MSE Ltd, London, England). To this emulsion 2.5 ml 18.7% defatted (5) bovine serum albumin (Sigma Chemical Company, St. Louis, Mo., USA), 2.0 ml 3.0 M NaCl and 2.0 ml distilled water were added. The mixture was then equilibrated for 30 min at 37°C. The assay system contained 150 µl of this mixture, enzyme and 0.01 M Tris HCl in 1.0 M NaCl in a total volume of 200 µl. After 15 min preliminary incubation at 37°C in a water bath, the tubes shaking at 50 strokes per minute, the enzyme was added. The reaction was stopped (usually after 15 min) by adding 3.25 ml of a mixture containing methanol, chloroform, heptane 1.41:1.25:1 (v/v/v). 1.0 ml 0.10 M potassium carbonate buffer, pH 10.5, was added (3) and the tubes were vigorously shaken and then centrifuged for 30 min at 1000 rpm in a Sorvall GLC 1 centrifuge (HL-4 rotor). Model experiments showed that 70% of the fatty acid soaps released were extracted into the upper phase. This extraction was not influenced by the various concentrations of NaCl used in the incubation system. 1.0 ml of the upper phase was transferred to a counting vial containing 5 ml Aquasol (NEN Chemicals GmbH, Dreieichenhain, Germany). Radioactivity was determined in a Model 3020 Packard liquid scintillation-spectrometer. When determining the pH optimum of the lipases the buffer of the assay mixture was replaced by distilled water and 40 µl of buffer pH 3.0–7.0 were added to the

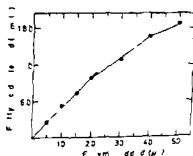


Fig. 2. Effect of the concentration of rat pharyngeal lipase on the amount of fatty acid released. The enzyme solution (prepared as described under Methods) was diluted 10-fold. Varying amounts were incubated in the routine assay system. The incubation time was 60 min.

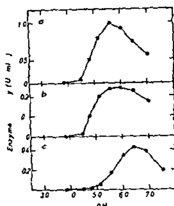


Fig 3 Effect of pH on the lipase activity The incubation system was prepared as described under Methods a 10 µl of enzyme solution (diluted 3 fold) prepared from rat tongue b 10 µl of esophageal aspirate from a human new born with congenital esophageal atresia c 10 µl of gastric contents from an infant with pyloric stenosis

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The use of a radioactive labelled substrate permits accurate determination of very low lipase activities Optimal assay conditions were worked out using a rat tongue extract as enzyme source The amount of fatty acid released was linear with time (Fig 1) and enzyme concentration (Fig 2) until about 100 nmoles had been released into the medium This corresponds to a 10% hydrolysis of the triglycerides Under the conditions used optimal activity of the rat pharyngeal lipase was found in the pH interval 5 0–6 0 (Fig 3*a*)

To study the composition of the products formed during various degrees of hydrolysis different amounts of enzyme were added to the incubation system (Fig 4) For the rat enzyme (Fig 4*a*) there was a rapid release of diglyceride and a much slower release of monoglyceride When the largest amount of enzyme was used diglyceride was still the major partial glyceride found

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Lipase activities of the esophageal aspirates were 0 5 and 1 3 U×ml⁻¹ respectively These activities are of the same magnitude as those found by Hamosh et al in human adults (10) and are in agreement with our previous observation that the ability to synthesize and secrete the pharyngeal lipase is already well developed in the newborn (9) The composition of the products formed during hydrolysis of emulsified triglyceride when either esophageal aspirate or gastric content were as

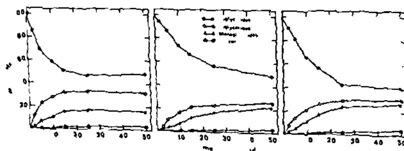


Fig 4 Composition of reaction products formed during hydrolysis of emulsified trioleate The incubations were performed as described under Methods Enzyme sources were varying amounts of (*a*) extract from rat tongue (*b*) esophageal aspirate (*c*) gastric contents

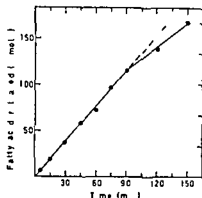


Fig 1 Time course of the release of fatty acids during incubation with rat pharyngeal lipase The enzyme solution (prepared as described under Methods) was diluted 6 fold 10 μl was incubated according to the routine assay procedure

mosh et al (10) found lipase activity in esophageal aspirates from healthy adult humans suggesting a pregastric origin also for the human lipase These authors suggested the name pharyngeal lipase which will be used in the present study

The aims of the present study were firstly to develop a convenient procedure for the determination of pharyngeal lipase activity using a long chain triglyceride as the substrate secondly to find out if the gastric lipolytic activity in the human newborn originates from pregastric tissues and if so thirdly to compare some properties of the human and the rat pharyngeal lipases

METHODS

Preparation of enzyme sources Sprague Dawley rats were killed under light ether anesthesia and the rear part of the tongues including the von Ebner glands were cut off and kept frozen until use This part of the tongue is rich in lipolytic activity (11 16) In each preparation 3–4 tongues were used The tongues were minced with scissors and homogenized in 10 volumes of 0 01 M Tris Hcl buffer pH 8 5 by the use of a Potter Elvehjelm homogenizer Homogenates were centrifuged for 30 min at 39000 g The pellets were rehomogenized in 10 volumes of 0 01 M Tris HCl in 1 0 M NaCl pH 8 5 and then centrifuged as described above The second supernatant was then used as source of rat pharyngeal lipase

Two fullterm infants with congenital esophageal atresia were investigated as follows before operative treatment aspirates of esophageal fluid were collected from the upper esophageal pouch The aspirates were immediately frozen and stored at −20 C until analyzed On operation a broncho-esophageal fistula was found in one infant and a tracheo esophageal fistula in the other The fluid obtained from the first infant was watery and alcaline pH 9 0 Where not otherwise stated the aspirate obtained from this infant has been used as the enzyme source in the experiments performed

Gastric contents were collected from a 25 days old boy suffering from pyloric stenosis The diagnosis was confirmed on operation The pH of the sample was 3 6 The sample was centrifuged for 15 min at 12000 g The clear phase below the upper fatty layer was used as enzyme source

Assay systems 2×10^7 cpm of purified (2) tri(9 10(n) ^{3}H) oleylglycerol (The Radiochemical Centre Amersham England) was mixed with 25 mg purified (70) olive oil 1 0 ml 10% gum arabic 3 0 ml 0 2 M sodium citrate buffer pH 6 0 and 2 0 ml distilled water were added The mixture was cooled with ice water and sonicated for 4 min at maximal effect in a 100 W disintegrator (MSE Ltd London England) To this emulsion 2 5 ml 18 7% defatted (5) bovine serum albumin (Sigma Chemical Company St Louis Mo USA) 2 0 ml 3 0 M NaCl and 2 0 ml distilled water were added The mixture was then equilibrated for 30 min at 37°C The assay system contained 150 μl of this mixture enzyme and 0 01 M Tris HCl in 1 0 M NaCl in a total volume of 200 μl After 15 min preliminary incubation at 37°C in a water bath the tubes shaking at 50 strokes per minute the enzyme was added The reaction was stopped (usually after 15 min) by adding 3 25 ml of a mixture containing methanol chloroform heptane 1 41 1 25 1 (v/v/v) 1 0 ml 0 10 M potassium carbonate buffer pH 10 5 was added (3) and the tubes were vigorously shaken and then centrifuged for 30 min at 1000 rpm in a Sorvall GLC 1 centrifuge (HL-4 rotor) Model experiments showed that 70% of the fatty acid soaps released were extracted into the upper phase This extraction was not influenced by the various concentrations of NaCl used in the incubation system 1 0 ml of the upper phase was transferred to a counting vial containing 5 ml Aquasol (NEN Chemicals GmbH Dreieichenhain Germany) Radioactivity was determined in a Model 3020 Packard liquid scintillation spectrometer When determining the pH optimum of the lipases the buffer of the assay mixture was replaced by distilled water and 40 μl of buffer pH 3 0–7 0 were added to the

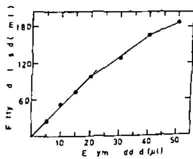


Fig 2 Effect of the concentration of rat pharyngeal lipase on the amount of fatty acid released The enzyme solution (prepared as described under Methods) was diluted 10-fold Varying amounts were incubated in the routine assay system The incubation time was 60 min

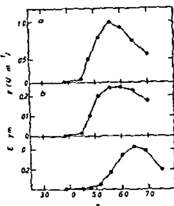


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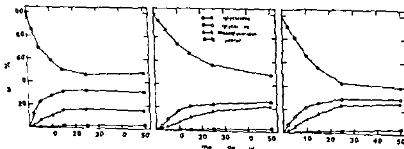


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Submitted Oct 29 1976
Accepted Dec 15 1976

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ROLE OF FEEDING ON LIPASE ACTIVITY IN GASTRIC CONTENTS

B FREDRIKZON and O HERNELL

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ABSTRACT Fredrikzon B and Hernell O (Department of Paediatrics and Department of Chemistry Section on Physiological Chemistry University of Umeå Umeå Sweden) Role of feeding on lipase activity in gastric contents Acta Paediatr Scand 66 479 1977.—Lipase activity was recorded in gastric contents collected from healthy term and preterm neonates In contrast to pancreatic lipase activity this lipase activity was higher at pH 5 5 than at pH 8 0 and it was more resistent to acid inactivation Lipase activity was found in gastric contents from all infants who were regularly fed but was not present in gastric contents from some infants when collected before regular feeding was established During test meals lipase ac tivity in gastric contents increased considerably in all infants studied During such a test meal there was a progressive relative decrease in triglycerides whilst diglycerides showed a relative increase suggesting an active lipolytic process in the stomach An assay procedure for deter mination of lipase activity in gastric contents is also described

KEY WORDS Gastric lipolysis lipase activity pharyngeal lipase lipid digestion neonatal period

Low activities of pancreatic lipase are found in intestinal contents from healthy infants during test meals (16) and intraluminal concentrations of bile acids are lower in infants as compared to adults (16 19) Two essential prerequisites for optimal digestion and absorption of lipids would appear to be incompletely developed at birth Consequently faecal fat excretion is often comparatively high during the first months of life (7) This functional immaturity of the newborn seems paradoxical since in the newborn nearly half of the consumed energy is supplied by dietary lipids (8) and has raised the question as to whether or not other lipolytic mechanisms may contribute to the digestion of lipids in infants (18) In a preliminary report milk lipids were readily hydrolyzed mainly to diglycerides and free fatty acids in the stomachs of infants with pyloric stenosis suggest ing a physiological role of the lipase activity in gastric contents (17)

In the present report gastric contents ob tained from healthy pre and fullterm infants in the fasting state and during test meals were studied This was done to find out if gastric lipase activity is already present at birth and if secretion of the lipase is stimulated by feeding

MATERIALS AND METHODS

Fasting subjects Sixteen infants gestational age (post menstrual) 33–41 weeks were investigated as follows before a scheduled meal a feeding tube was introduced into the stomach and by gentle suction about one ml of gastric contents was collected Samples from 10 subjects were obtained before their first meal The other six in fants had already been fed cow s milk formula pasteur ized human milk or were breastfed

Test meals Six infants gestational age (postmenstrual) 33–37 weeks were at the time for a scheduled feeding given a test meal of pasteurized human milk 20–30 ml/kg body weight Samples of gastric contents were collected before and at intervals during the test meal Lipid com position of gastric contents was analyzed in samples ob tained during one test meal and samples from the others were used for the determination of lipase activities

Table 1 *Clinical data on subjects investigated and laboratory findings in fasting subjects and infants with pyloric stenosis*

Cases	Gest age (weeks)	Birth weight (g)	Age (days)	Food intake	pH of gastric aspirate	Gastric aspirate (ml)	Lipase activity (U×ml⁻¹)	Comments
Fasting subjects								
LT 1 } twins	39	2 930	1	–	3 3		54 4	Midline cervical cleft
LT 2 } twins	39	? 860	1	–	2 9		76 0	Postnatal asphyxia
HN	39	3 480	1	–	2 3		23 1	Normal
LD	40	3 250	1	–	2 5		25 5	Normal
DN	40	4 370	2	+?	2 3		2? 9	Normal
LM	41	3 660	3	+	2 ?		15 3	Normal
MN	38	3 480	6	+	2 0		55 1	Normal
SN	36	2 190	16	+	3 8		?6 8	Spermatic cord torsion
FG 1 } twins	33	1 760	32	+	2 7		25 4	Caesarean section
FG 2 } twins	33	1 880	32	+	2 6		2 6	Caesarean section
Pyloric stenosis								
JN	40	3 890	26	+	4 4	21	48 5	Operated
JN	40	3 410	36	+	4 6	9–23	34 3	Operated
ÖG	36	2 700	69	+	2 6	36–1?0	32 6	Not operated
Test meals								
HM	32	1 740	18	+	Lipid composition of gastric contents is shown in Fig 4			
HN	32	1 950	20	+	pH and lipase activity of gastric contents collected during the test meals are shown in Fig 3			
SM	36	? 3?0	?0	+				
HT	37	2 550	2	+				
SE	33	1 500	25	+				Light for-date
KN	36	1 640	38	+				

is easily dispersed in an aqueous medium by mechanical stirring alone and thus there is no need for any exogenous emulsifier Though the substrate itself is practically insoluble in water the liberated butyric acid is readily water soluble During incubations performed according to the standard assay procedure the release of butyric acid was linear with time and enzyme concentration Sometimes a progressive loss of enzyme activity was noted during incubations performed in the absence of bile acids (Fig 1 experiment (c)) However linear kinetics were always obtained when sodium taurocholate was added to the medium (Fig 1 experiment (*d*)) At pH 8 0 virtually no lipase activity was found in gastric contents irrespective of presence or absence of bile acids (Fig 1 experiments (*a*) and (*b*))
Reproducibility of the assay procedure was tested by determination of the lipase activity in one sample on 10 different occasions during a three months period resulting in a mean=32 6 and S D =1 5 (4 6%) Thus lipase activity was unaffected by several freezings and thawings and remained stable at 4°C for hours even at as low a pH as 2 6

Duodenal juice lipase activity at pH 5 5 was always lower than that at pH 8 0 (Fig 2) When the pH of duodenal juice was adjusted to 2 5 virtually all lipase activity was lost within five minutes

Clinical material

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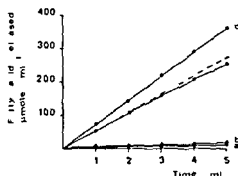


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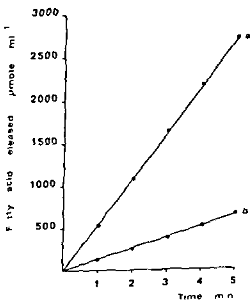


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Fasting subjects								
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LT 2 } twins	39	2 860	1	−	?9		76 0	Postnatal asphyxia
HN	39	3 480	1	−	2 3		23 1	Normal
LD	40	3 250	1	−	2 5		25 5	Normal
DN	40	4 370	2	+?	2 3		2? 9	Normal
LM	41	3 660	3	+	2 2		15 3	Normal
MN	38	3 480	6	+	2 0		55 1	Normal
SN	36	2 1?0	16	+	3 8		?6 8	Spermatic cord torsion
FG 1 } twins	33	1 760	32	+	2 7		25 4	Caesarean section
FG 2 } twins	33	1 880	32	+	2 6		2 6	Caesarean section
Pyloric stenosis						Gastric aspirate (ml)		
I?	40	3 890	26	+	4 4	21	48 5	Operated
IN	40	3 410	36	+	4 6	9– 23	34 3	Operated
ÖG	36	? 700	69	+	?6	36–120	32 6	Not operated
Test meals								
HM	32	1 ?40	18	+	Lipid composition of gastric contents is shown in Fig 4			
HN	32	1 950	?0	+	pH and lipase activity of gastric contents collected during the test meals are shown in Fig 3			
SM	36	2 3 0	0	+				
HT	37	2 550	2	+				
SE	33	1 ?00	25	+				Light for-date
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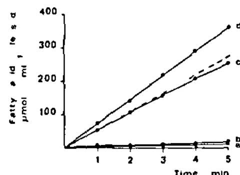


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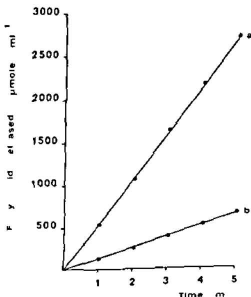


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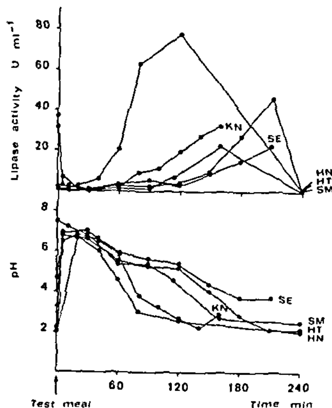


Fig 3 Variation of pH and lipase activities of gastric contents during test meals Samples were collected from infants according to Table 1

into the stomach The other sample was mucous (pH=7 4) and no lipase activity was found (see Table I and Fig 3)

Soon after administration of test meals the pHs of the gastric contents rose to around 6 5 During the following hours the pHs gradually decreased towards values found before the test meals (Fig 3)

The relative molar concentrations of lipids in gastric aspirates during one test meal showed a progressive decrease of triglycerides and an increase mainly of diglycerides (Fig 4) In this particular experiment a molar concentration of about 35% of diglycerides was found 2½ hours after administration of the test meal

Pyloric stenosis All samples from these infants were grossly mixed with milk and the volumes collected were greater than those from healthy infants Lipase activities were found in all samples of gastric contents from these infants (Table I)

fants on their first day of life and before their first meal

Test meals To further study the influence of the feeding process on enzyme activity it was decided to follow the lipase activity of gastric contents during test meals During the meals lipase activities were often initially low but in all infants a considerable increase was found after two to three hours Thereafter lipase activity was again decreased and could not be measured after four hours in three infants (Fig 3) To exclude the possibility of interference from pancreatic lipase activity that might have regurgitated into the stomach those samples containing the highest activities in each subject were assayed both at pH 5 5 and at pH 8 0 At pH 8 0 virtually no lipase activity was found in any of these samples Samples collected from two infants before the test meal were not representative of gastric contents one sample was bile stained (pH=4 7) and at pH 8 0 higher activity was found than that at pH 5 5 suggesting reflux of duodenal contents

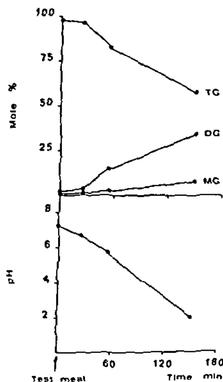


Fig 4 pH of gastric contents and molar composition of glycerides during a test meal procedure Gastric contents were collected at intervals and analyzed as described previously (17) TG DG and MG stand for triglyceride diglyceride and monoglyceride respectively

DISCUSSION

Since regurgitation into the stomach of intestinal juice containing pancreatic lipase activity could not be excluded in the present study it seems justified to discuss briefly some of the characteristics of the pancreatic lipase Pancreatic lipase hydrolyzes dietary triglycerides present in the intestine in an emulsified state essentially to 2 monoglycerides and free fatty acids (2 14) When doudenal juice lipase activity is determined against tributyrin optimal activity is found around pH 8 (3) and considerably lower activities are recorded at pH 5 5 (Fig 2) The activity of pancreatic lipase is rapidly lost at pH 3 or below (5 9) The cholesterol ester hydrolase (pancreatic esterase) (13) is more active against micellar or water soluble substrates e g *p* nitrophenyl acetate (15)

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In infants with pyloric stenosis there should be a reduced risk for duodenal juice reflux into the stomach and thus for contribution of pancreatic lipase activity The gastric juice lipase activities recorded in these infants were however of the same magnitude as those for healthy infants

Thus there are several properties of lipase activity in gastric contents which differ from those of pancreatic and duodenal juice lipase activity This makes it very unlikely that regurgitation of pancreatic lipase activity into the stomach should make a major contribution to lipase activities recorded in gastric aspirates

In fasting subjects lipase activities in gastric contents varied However there was no obvious relation between the clinical condition of the neonate i e gestational age postnatal age birth weight or pH of the gastric aspirate and the lipase activity recorded All samples devoid of lipase activity were collected from healthy fullterm infants on their first day of life before the establishment of a feeding routine However all infants responded to test meals by a considerable increase of lipase activities and in fact the highest value recorded in this study was found in a preterm girl (case HN Fig 3) indicating that the ability to secrete this lipase is already well developed in neonates The stimulatory effect of food was also noted in infants with pyloric stenosis lipase activities comparable to those found in gastric contents from healthy neonates were recorded in these infants despite gross dilution of specimens with partly digested milk A physiological role for this lipase is supported by the increase of lipase activity during the digestive period The relationship between lipase activity and food intake has not been properly recognized in previous reports of gastric juice lipase

The importance of gastric lipolysis in the digestion of lipids is however still uncertain Since milk lipids are readily hydrolyzed by gastric juice lipase but serve as poor substrate for the pancreatic lipase it has been suggested that the importance of gastric lipolysis is limited to the infantile period of life (5) However efficiency of fat digestion is not only a matter of enzyme activities but also of solubilization of the dietary lipids and the lipid products formed It has recently been proposed that the amphiphilic properties of the products formed during gastric lipolysis may facilitate the dispersion and emulsification of dietary lipids (10) If this is true gastric lipolysis may serve as a complement to the normal mechanisms of lipid digestion in two ways

1 By increasing the net hydrolysis of milk lipids

2 By facilitating the dispersion and emulsification of dietary lipids

The first statement gains support in the

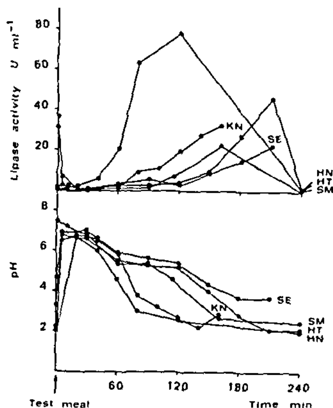


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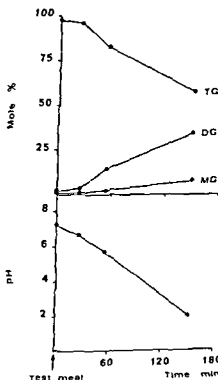


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present study for the following reasons the gastric lipase activity is well developed al ready at birth and this lipase activity increases during feeding Finally during digestion of milk in the stomach there is a progressive rel ative decrease of triglycerides and a relative increase of diglycerides, suggesting an active lipolytic process

Although the second statement is more speculative, there are some experimental data supporting this idea (18)

ACKNOWLEDGEMENTS

This work was supported by grants from Semper Fund for Nutritional Research the Swedish Nutrition Founda tion the Swedish Medical Research Council (B-03X 00727) and the Medical Faculty University of Umeå

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Submitted Oct 29 1976
Accepted Dec 15 1976

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Acta Pædiatr Scand 66 485–487 1977

THE FOAM STABILITY TEST ON GASTRIC ASPIRATE IN THE PREDICTION OF RESPIRATORY DISTRESS SYNDROME

M E SPEER A J S CORBET P FLAX and A J RUDOLPH

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ABSTRACT Speer M E Corbet A J S Flax P and Rudolph A J (Department of Pediatrics Baylor College of Medicine Houston Texas USA) The foam stability test on gastric aspirate in the prediction of respiratory distress syndrome Acta Paediatr Scand 66 485 1977 —To evaluate the usefulness of the foam stability test (FST) on gastric aspirate for predicting respiratory distress syndrome (RDS) in premature infants samples were col lected at delivery or within 30 min from 194 infants ≤36 weeks gestation Of 123 samples adequate for complete testing 44 were positive at 1 2 dilution 43 were positive only at dilu tions <1 2 and 36 were negative at all dilutions RDS was found in 2% 21% and 25% of each group respectively The FST on gastric aspirate at birth gives useful information only if posi tive at 1 2 when a very low incidence of RDS may be expected However a large proportion of infants with FST negative at 1 1 do not develop RDS and hence the test is of limited value in screening for those with highest risk

KEY WORDS Respiratory distress syndrome foam stability test premature infants

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Submitted Oct 29 1976
Accepted Dec 15 1976

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Acta Pædiatr Scand 66 485–487 1977

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Submitted Nov 8 1976
Accepted Nov 25 1976

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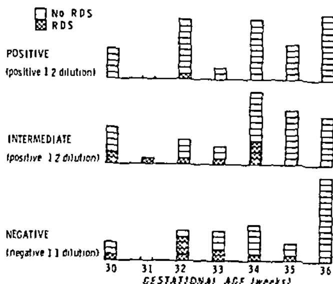


Fig. 1. Relationship of the foam stability test to the diagnosis of respiratory distress syndrome and gestational age.

repeated after thawing at 24, 48, 72 and 168 hours of storage.

The clinical criteria for the diagnosis of RDS were 1) typical diffuse granular densities on the chest radiograph and 2) maximal inspired oxygen greater than 50% (3).

RESULTS

Of the 194 samples collected, 45 (23%) were of insufficient volume to perform the FST. A further 26 (13%) samples had a negative FST at a 1:2 dilution but were of inadequate volume to be tested at lower dilutions. Both of these groups were excluded from the analysis.

Of the 123 samples adequate for an FST, 44 were positive at a dilution of 1:2, 43 were positive only at a dilution <1:2 (intermediate) and 36 were negative at all dilutions. Fig. 1 shows the relationship of the FST to gestational age and the diagnosis of RDS. RDS was found in 2%, 21% and 25% of each group respectively. The incidence of RDS was significantly lower in infants with an FST positive at 1:2 dilution when compared to infants with FST negative at all dilutions or positive only at dilutions <1:2 ($p<0.01$). The incidence of RDS in those with an FST positive at a dilution of <1:2 was similar to that in infants with a negative test.

The gastric aspirate pH of the 123 samples ranged from 3.5 to 8.0 with a mean of 6.7.

In the pooled control sample of gastric aspirate there was no change in the positive FST when tested after freezing for 24, 48, 72 and 168 hours.

DISCUSSION

The pH determinations suggest that gastric aspirate obtained in this study represented amniotic fluid and/or swallowed tracheal fluid and was a suitable fluid for the FST (1).

From the analysis of all investigations carried out so far using amniotic fluid, it can be concluded that a positive FST at 1:2 dilution is associated with an extremely low risk of RDS (3, 8, 9, 10, 12, 14). The present results for the FST in gastric aspirate are in complete agreement because only 2% of infants in our series developed RDS when the FST was positive at 1:2 dilution. In addition, all authors have found that, although intermediate test results in amniotic fluid may correlate well with an intermediate L/S ratio, the development of RDS in this group cannot be predicted with an acceptable degree of accuracy (3, 8, 9, 12, 14). Our results clearly suggest this to be true also of the FST in gastric aspirate.

The data in the literature concerning a negative FST are, however, conflicting. Clements et al., using amniotic fluid, found that a negative test at 1:1 dilution was associated with a very high risk of RDS (3), but others have not found such a close relationship (9, 10, 12, 14). Recently Cowett et al. (4), utilizing the FST performed with gastric aspirate, have suggested there is excellent correlation between a negative FST and the development of RDS. However, our results and those of Evans et al. (6) more closely agree with the majority of workers utilizing the FST on amniotic fluid in that many negative tests were obtained in infants who subsequently did not develop RDS. This finding is consistent with the view that the development of lung maturity occurs prior to the expression of this maturity in the FST on amniotic fluid or gastric aspirate.

An alternative explanation is that before the

FST is performed substances capable of promoting stable foam are degraded or inactivated this would appear unlikely since our pooled sample showed no change in the positive FST with storage for up to one week

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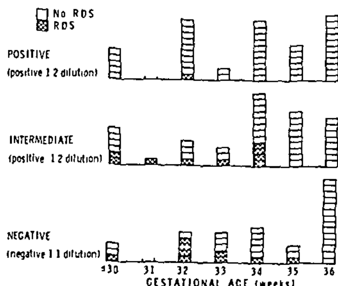


Fig 1 Relationship of the foam stability test to the diagnosis of respiratory distress syndrome and gestational age

repeated after thawing at 24 48 72 and 168 hours of storage

The clinical criteria for the diagnosis of RDS were 1) typical diffuse granular densities on the chest radiograph and 2) maximal inspired oxygen greater than 50% (3)

RESULTS

Of the 194 samples collected 45 (23%) were of insufficient volume to perform the FST A further 26 (13%) samples had a negative FST at a 1 2 dilution but were of inadequate volume to be tested at lower dilutions Both of these groups were excluded from the analysis

Of the 123 samples adequate for an FST 44 were positive at a dilution of 1 2 43 were positive only at a dilution <1 2 (intermediate) and 36 were negative at all dilutions Fig 1 shows the relationship of the FST to gestational age and the diagnosis of RDS RDS was found in 2% 21% and 25% of each group respectively The incidence of RDS was significantly lower in infants with an FST positive at 1 2 dilution when compared to infants with FST negative at all dilutions or positive only at dilutions <1 2 ($p<0\ 01$) The incidence of RDS in those with an FST positive at a dilution of <1 2 was similar to that in infants with a negative test

The gastric aspirate pH of the 123 samples ranged from 3 5 to 8 0 with a mean of 6 7

In the pooled control sample of gastric aspirate there was no change in the positive FST when tested after freezing for 24 48 72 and 168 hours

DISCUSSION

The pH determinations suggest that gastric aspirate obtained in this study represented amniotic fluid and/or swallowed tracheal fluid and was a suitable fluid for the FST (1)

From the analysis of all investigations carried out so far using amniotic fluid it can be concluded that a positive FST at 1 2 dilution is associated with an extremely low risk of RDS (3 8 9 10 12 14) The present results for the FST in gastric aspirate are in complete agreement because only 2% of infants in our series developed RDS when the FST was positive at 1 2 dilution In addition all authors have found that although intermediate test results in amniotic fluid may correlate well with an intermediate L/S ratio the development of RDS in this group cannot be predicted with an acceptable degree of accuracy (3 8 9 12 14) Our results clearly suggest this to be true also of the FST in gastric aspirate

The data in the literature concerning a negative FST are however conflicting Clements et al using amniotic fluid found that a negative test at 1 1 dilution was associated with a very high risk of RDS (3) but others have not found such a close relationship (9 10 12 14) Recently Cowett et al (4) utilizing the FST performed with gastric aspirate have suggested there is excellent correlation between a negative FST and the development of RDS However our results and those of Evans et al (6) more closely agree with the majority of workers utilizing the FST on amniotic fluid in that many negative tests were obtained in infants who subsequently did not develop RDS This finding is consistent with the view that the development of lung maturity occurs prior to the expression of this maturity in the FST on amniotic fluid or gastric aspirate

An alternative explanation is that before the

Acta Pædiatr Scand 66 489–493 1977

EFFECT OF FARLY AND LATE CORD CLAMPING ON THE SYSTOLIC TIME INTERVALS OF THI NEWBORN INFANT

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ABSTRACT Yao A C and Lind J (Departments of Paediatrics Karolinska Institute Stockholm Sweden and Downstate Medical Center State University of New York USA) Effect of early and late cord clamping on the systolic time intervals of the newborn infant Acta Paediatr Scand 66 489 1977 —The effect of early and late cord clamping on the left ventricular performance of the newborn infant was assessed by measuring the systolic time intervals from the indirect carotid pulse tracings and simultaneous phonocardiogram and electrocardiogram The study was performed in 13 normal full term infants sequentially at 20–105 min 6–6½ hrs and 24–27 hrs of age The umbilical cords were clamped early in 7 (E C) and clamped late in 6 infants (L C) The ratio of the pre-ejection period (PEP) to the left ventricular ejection time (LVET) was found to be significantly higher in the L C infants (mean±S E 0 400±0 18 0 433±0 018 0 410±0 021) compared with those of the E C (0.334±0 010 0.347±0 009 0.361±0 007) with *p* values of <0 01 <0 005 and <0 05 respec ti ely in the three examination periods This higher PEP/LVET ratios in the L C infants were mainly attributable to a prolongation of the PEP It is suggested that late cord clamping by allowing a sizable placental transfusion appeared to affect adversely the left ventricular performance of the neonate Furthermore it is suggested that in evaluating systolic time intervals of the neonate during the first days of life the volumic state or status of placental transfusion should be taken into consideration as a determinant

KEY WORDS Newborn placental transfusion left ventricular function

The size of placental transfusion is important in the evaluation of the newborn infant in the immediate transitional period after birth be cause of its effects on the circulatory respira tory urinary and other systems of the body (18) It has been shown that by delaying the clamping of the umbilical cord at birth for 3 min or more the blood volume of the infant may be increased by 30% or more Thus the blood volume of the neonate at birth can vary from 77 ml/kg to as much as 120 ml/kg (10) The late-clamped infant adjusts to the larger blood volume by hemoconcentration rapid plasma extravasation and increased urinary output during the first hours of life Consider able differences have been shown in the physiological adaptation to extra uterine life between the early and late clamped infant and no clear cut advantages have been shown in the infant with the larger blood volumes (18) The late-clamped infant was found to sustain a higher systemic blood pressure systemic vascular resistance as well as higher pulmonary artery pressure during the first days of life (1 2 11) The purpose of this study was to determine by a non invasive technique the effect of placental transfusion on the left ventricular function of the newborn infant

MATERIALS AND METHODS

The subjects were 13 normal full term infants of healthy mothers whose pregnancies and deliveries were uncom plicated by any pathology The gestational ages ranged from 37 to 42 weeks weight from 4 0 to 4 750 g Umbili

ences in heart rate QS_2 or LVET The PEP/LVET ratios at all three age periods examined (mean ages 50 min 6 hrs and 24 hours old) showed significantly higher values in the L C group (Mean±S E 0 400±0 18 0 433±0 009) 0 410±0 052 and in the E C group 0 334±0 026 0 347±0 025 0 361±0 007) with p values of <0 01 <0 005 and <0 05 respectively (Fig 2) These elevated PEP/LVET ratios in the L C group were unrelated to the heart rate or LVET but are due to a prolongation of the PEP

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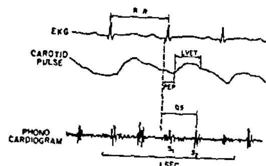


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L C	126 0	289 83	207 16	82 66
	2 44	2 55	3 76	2 55
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	5 62	8 37	6 73	1 99
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	4 60	5 46	2 51	5 45
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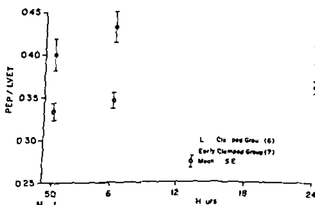


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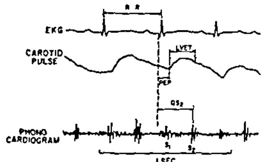


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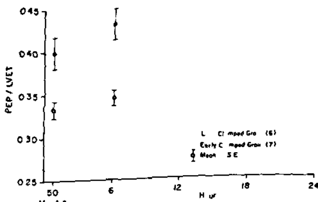


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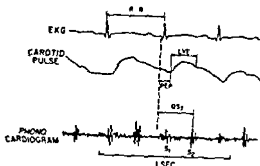


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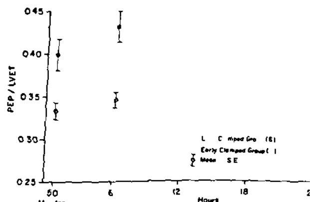


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DISCUSSION

The PEP/LVET ratio derived from the carotid pulse in the normal full term infant during the first 3 days of life had been reported to be 0 390 0 430 and 0 370 respectively (7) These values are slightly lower but fairly close to those of our L C group They are higher than those of the E C group However the time of cord clamping in the newborn was not noted in that report nor in those of others (7 14)

It is interesting to compare our PEP values with those of others using different techniques (Table 3) Using the Doppler Ultrasound the average PEP in term fetuses during labour were 70 ms and 73 ms (9 13) These values are very close to those of our E C infants which may overlap the upper limits of normal in adults By echocardiographic technique the

Table 3 *Comparison of systolic time intervals in term infants by different techniques*

CP carotid pulse DUS Doppler ultrasound Echo echocardiography ms millisecond E C early cord clamping L C late cord clamping

Author	Age	PEP (ms)	PEP/LVET	Method
Hedvall	0–23 h	80	0 39	CP
	24–48 h	84	0 43	
	2–3 m	68	0 35	
Murata et al	Term fetus 38–40 weeks	70		DUS
Organ et al	Term fetus	73		DUS
Riggs et al	0 75–24 h	60–95	0 32–0.53	Echo
Yao & Lind	50 min E C	69 9	0 33	CP
	L C	82 7	0 40	
	6 hrs E C	72 6	0 35	
	L C	98 0	0 43	
	24 hrs E C	75 7	0 36	
	L C	83 5	0 41	

PEP of the left ventricle was found to range from 60 to 95 ms during the first day of life (15) Although the time of cord clamping was not known in the latter study the PEP values as well as the PEP/LVET ratios spanned a range corresponding those of our E C and L C infants These authors noted that 20% of their cases have the higher values similar to those of our L C group These values in the newborn infant are by and large higher than those of older infants and adults (4 6 16) suggesting strained left ventricular function

The blood volume of the fetus at term (17) is about that of the E C infant (18) The increase in blood volume after late cord clamping is sizable and abrupt This usually occurred in an arbitrary manner at birth In no other situation in life does this physiologic phenomenon occur in such an abrupt manner that a transfusion of as much as 100 ml of blood can occur within 3 minutes to a 3½ kg infant Its physiologic consequences on the neonate s early extra uterine adaptation was shown to be significant The L C infant appeared to take a longer time and exert more effort to achieve extra uterine adjustment especially in its cardiorespiratory

Table 1 *Clinical data of newborn infants studied*

Group	No of cases	Body weight (g) Mean±S D	Length (cm) Mean±S D	Time of cord clamping Mean
I	7	3 451 4±562	50 6±1 8	1.5 sec
II	6	3 503 3±632	50 5±2 3	4 min

cal cords were clamped early (E C) at 1–3 sec after birth in 7 and clamped late (L C) at 3–5 min in 6 infants (Table 1) Simultaneous electrocardiogram (ECG) phonocardiogram (PCG) and indirect carotid pulse traces (CP) were recorded in each infant sequentially at 20–105 min 6–6½ hrs and 24–27 hrs of life The infants were warmly clad and room temperature kept constant within the range 26 5 to 28 5 C The CP was recorded by an arterial pulse receptor made of plastic connected to an Elema Schönander EMT510C transducer (0 5–30 Hertz frequency response) by an air filled tube The receptor was placed gently over the infant s carotid area with its head slightly turned to the opposite side and in mild extension Tracings were made after oscilloscopic display of the CP were noted to be stable and acceptable at a paper speed of

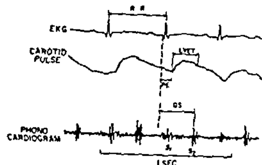


Fig 1 Simultaneous recordings of lead II ECG phonocardiogram and carotid pulse showing how the different systolic time intervals are measured

100 mm/sec in an Elema Schonander Mingograf 81 An EMT 25 microphone was used for the PCG recording over the lower left sternal border and lead II ECG taken by standard technique

Systolic time intervals (STI) were measured as follows (Fig 1) (i) electromechanical systole (QS_2) from the onset of QRS complex to the first high frequency deflection of second heart sound (ii) left ventricular ejection time (LVET) from the beginning of the upstroke of carotid pulse to the dicrotic notch (iii) pre-ejection period (PEP) derived by subtracting LVET from QS_2 thereby eliminating the delay in transmission of the arterial pulse to the carotid artery The PEP/LVET ratio was then calculated The R R interval from the ECG was measured for heart rate The average of 10–15 heart beats was analysed for each period of study in each infant

Table 2 *Heart rate and systolic time intervals of early and late clamped infants*

HR heart rate LVET left ventricular ejection time PEP pre-ejection period QS_2 electromechanical systole E C early clamped group L C late clamped group Values in Mean±S E

Group	HR (beat/min)	QS_2 (ms)	LVET (ms)	PEP (ms)
At age 50 min				
E C	125 00 5 88	279 85 9 77	210 0 7 85	69 85 2 56
L C	126 0 2 44	289 83 2 55	207 16 3 76	82 66 2 55
p value	NS	NS	NS	<0 005
At age 6 hrs				
E C	115 43 7 29	278 85 7 51	209 0 6 69	72 57 2 51
L C	110 67 4 60	295 17 8 49	206 16 6 83	98 0 3 36
p value	NS	NS	NS	<0 005
At age 24 hrs				
E C	117 57 5 62	285 42 8 37	209 71 6 73	75 71 1 99
L C	115 83 4 60	287 0 5 46	203 0 2 51	83 50 5 45
p value	NS	NS	NS	<0 100

RESULTS

A significantly longer PEP was noted in the L C group than in the E C group from birth to 6 hours and almost through 24 hours of life (Table 2) There were no significant differ

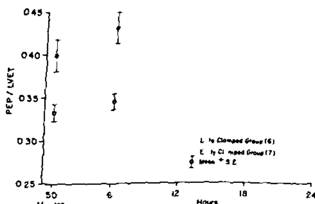


Fig 2 Mean PEP/LVET ratios of 7 early-clamped and 6 late-clamped full term infants showing significant differences at less than an hour of life ($p<0\ 01$) at 6 hours ($p<0\ 005$) and at 24 hours ($p<0\ 05$)

15 Riggs T Hirschfeld S Bormuth C Fanaroff A & Liebman J Neonatal circulatory changes An echocardiographic study To be published
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Submitted Nov 9 1976
Accepted Dec 1 1976

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A prolongation in the PEP accompanying abnormal fetal heart rate pattern had been recorded in human term fetuses in distress by Doppler cardiogram (9) Experiments in fetal lambs showed prolongation of the PEP upon occlusion of the cord and a shortening on its release (12) This change was ascribed to change in arterial pressure The high PEP/LVET ratio in the L C infants was due mainly to the prolongation of the PEP suggesting that this might be due to an increase in the afterload on the heart The systemic blood pressure has been observed to be higher in the L C than in the E C infants during the first 6 hours of life and the systemic vascular resistance likewise during the first day (1 11) However the diastolic arterial pressure needed to be measured in both E C and L C infants The rapid hemoconcentration observed in the L C infants (10 18) may have contributed to the increase in afterload on the heart The transiently higher left atrial pressure in the L C infant (3) may have increased the preload on the heart which should have increased stroke volume and LVET and decreased PEP Instead the opposite effect on the STI was seen in the L C infants suggesting that either the preload increase is too transient and insignificant because of rapid plasma extravasation or that there is an inherent contraction limit in neonatal myocardium Structural and functional studies of fetal and neonatal myocardium in lambs suggested the existence of such an inherent limitation (5 8) It is possible that the newborn myocardium in the human infant may have similar characteristics which limits its capacity to respond to an increased blood volume

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Acta Pædiatr Scand 66 495–500 1977

EFFECT OF AGE ON HUMAN ADIPOSE TISSUE METABOLISM AND HORMONAL RESPONSIVENESS

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ABSTRACT Nyberg G Hager A and Smith U (Department of Medicine II Sahlgren s Hospital University of Gothenburg and Department of Paediatrics University Hospital Linkoping Sweden) Effect of age on human adipose tissue metabolism and hormonal responsiveness Acta Paediatr Scand 66 495 1977.—The effects of age and fat cell size on the metabolism of white human adipose tissue were analysed independently Abdominal subcutaneous adipose tissue specimens were obtained from children varying in age from 0–15 years and from adults (mean age±S D 38.2±14 1 years) The basal rates of lipolysis and glucose incorporation into lipids were considerably higher in children than in adults even when differences in fat cell size had been taken into account Lipolysis in response to a maximal concentration of noradrenalin was higher in fat cells from children However on a percentage basis the responsiveness to this agent was similar in children and adults Irrespective of age glucagon did not elicit a lipolytic response Thus it does not seem that the increased lipolysis known to occur *in vivo* in the neonatal period is due to a direct effect of glucagon on white adipose tissue However the overall lipolytic capacity is increased in fat cells from children

KEY WORDS Fat cell metabolism age lipolysis glucagon catecholamines

The human infant obtains a large part of its energy requirements from fatty acids mobilized from both brown and white adipose tissue (24 28) Novak et al (22) have shown that lipid mobilization from white adipose tissue is particularly high during the first hours after birth and then declines rapidly During infancy the lipolytic rate is only slightly higher than in adults (22) This interpretation is however somewhat hampered by the fact that fat cell size of white adipose tissue increases rapidly during the first year of life (15 23) A tissue with a small fat cell size contains a larger number of fat cells per unit weight than a tissue with larger cells Since the data of Novak et al (22) were expressed per unit tissue weight rather than in terms of the cellularity the metabolic rates may be misleading

In a recent study on rat tissue the effects of fat cell size and age on adipocyte metabolism were analysed independently (14) It was found that irrespective of cell size the metabolic rates were considerably increased in the younger animals A pronounced difference in the cellular responsiveness to glucagon was found in that fat cells from young animals responded briskly to glucagon while adipocytes from older animals were glucagon resistant (14 21) These findings are in agreement with a recent report by Cooper & Gregerman (7) showing a decline in glucagon stimulated adenylate cyclase activity with age in rat fat cells

It is well-established that adult human fat cells respond only sluggishly (2) or not at all to the lipolytic effect of glucagon *in vitro* (5) In

view of the findings discussed above it may well be then that fat cells from young individuals are reponsive to glucagon Since glucagon levels are elevated in newborns (20) it seems of importance to evaluate the possible physiological significance of this agent for lipid mobilization

In the present investigation glucose incorporation and basal and stimulated lipolysis were evaluated in human adipose tissue with respect to both fat cell size and age

MATERIALS AND METHODS

Biopsies of abdominal subcutaneous adipose tissue were obtained from 32 children varying in age from a few hours up to 15 years The children were generally operated upon for inguinal hernia or retained testis Two newborns were included who were operated upon for esophagus atresia or an ovarian cyst The metabolic responses of fat cells from children were compared with the results obtained with abdominal subcutaneous fat from adults who were selected so as to have similar fat cells sizes as the children The adults were operated upon for cholecystolithasis (1) gastric ulcer (2) varicocele (3) ovarian cyst (2) renal stones (1) or a hyperplastic prostate (1)

After excision smaller biopsies weighing about 20–30 mg each were prepared and preincubated for 30 min in the same medium as was later used for the incubations The biopsies were then placed in fresh medium and incubated for 2 hrs at 37 C The incubation medium used was medium 199 (Statens Bakteriologiska Laboratorium Stockholm) modified to a glucose concentration of 1 0 mM containing 40 g/l bovine albumin (Fraction V Sigma Chemical Co St Louis Mo) and the indicated concentrations of noradrenalin (noradrenalin bitartrate Astra AB Sodertalje Sweden) or glucagon (Novo A/S Copenhagen) In some experiments 0 15 μCi [1 ^{14}C]glucose (New England Nuclear Corp Frankfurt/Main W Germany) was added to the incubation medium and the incorporation into the triglycerides determined The release of glycerol to the incubation medium was determined enzymatically (19) and taken as an index of lipolysis

After the incubation period the tissue lipids were extracted with chloroform/methanol (2 1 v/v) as described by Folch et al (9) An aliquot of the chloroform phase was taken for the determination of the radioactivity in the triglycerides using a Packard Tri-Carb liquid scintillation spectrometer (Packard La Grange Illinois) Glyceride glycerol was determined on the chloroform phase as described by Carlson (6) After saponification the fatty acids were in some experiments extracted with *n* heptane and the radioactivity determined

The metabolic parameters studies were expressed in terms of the cellularity of the specimens as previously suggested (1 12 25) *Fat cell size was determined after* isolation with collagenase as previously described (27) The amount of collagenase used was the same for both children and adults 5 mg per 3 ml medium The error of the sizing procedure used has been found to be 3 4% (27) Mean cellular volume was calculated as described by Goldrick (10) and mean cellular weight according to Hirsch & Gallian (13) assuming that the density of fat cells equals that of triolein When the triglyceride content of the biopsies and the mean cellular weight are known *the number of fat cells can be calculated*

Since adipocyte size is an important parameter for metabolic rates (1 16 17 25 29) the measured parameters were related to fat cell surface area calculated from the measured diameters as described by Zinder & Shapiro (29)

RESULTS

The subjects were divided into four groups of equal size according to the age of the donors (below 1 yr 1–4 yrs, 5–15 yrs and adults= 38 2±14 1 years mean ±S D)

Cell size

The correlations between basal lipolysis basal rate of glucose incorporation into triglycerides and cell surface area were significant for both adults and children between 5 and 15 years (*r* values between 0 581 and 0 828 $p<0\ 05$) For the children below 1 year and between 1–4 years however the correlation coefficients did not reach statistical significance

Age

Since cell size influenced the rates of metabolism this factor had to be eliminated in order to analyse the effect of age This was performed by multivariate analysis with weighted means (18) As shown in Table 1 adults had lower rates of glucose incorporation and basal *and stimulated lipolysis than the children* The differences were highly significant for all parameters measured for children in the two oldest age groups For the youngest children however only a tendency to a difference ($p<0\ 10$) was found for glucose incorporation

Between the three child groups there were no significant differences in the parameters measured except that basal lipolysis was lower in the youngest group *In this group one* specimen was obtained *from a child within 24* hrs after birth and in another child after 120

Table 1 *Metabolic variables in relation to age*

Figures within parentheses denote number of donors

Variable studied (nmoles/10[5] cells)	Variable held constant cell surface ($n \times 10^{-3}\ \mu m^2$)	Variable in relation to age (in years) <1	1–4	5–15	Adults[a] (38 2±14 1)
Basal glycerol release	≤14 0	37 5 (6)	59 7 (?)	49 2 (5)	17 3 (2)
	>14 0 ≤18 0	34 1 (?)	68 0 (5)	48 3 (4)	14 0 (3)
	>18 0	48 0 (2)	86 4 (5)	102 4 (1)	2? 4 (5)
		39 6 (10)	70 7 (12)	65 4 (10)	18 1 (10)
		<0 05 (<1 vs 1–4)	n s (1–4 vs 5–15)	<0 01 (5–15 vs adults)	
		n s (<1 vs adults)			
			<0 001 (1–4 vs adults)		
Glycerol release +5 0×10[-4] M noradrenalin	≤14 0	–	–	84 9 (?)	39 2 (2)
	>14 0 ≤18 0	–	147 5 ( )	73 3 (?)	29 7 (3)
	>18 0	165 8 (1)	179 6 (?)	149 7 (1)	38 0 (5)
		165 8 (1)[b]	138 0 (4)	114 0 (5)	34 1 (10)
			n s (1–4 vs 5–15)		
			<0 001 (1–4 vs adults)		
				<0 01 (5–15 vs adults)	
Basal incorporation of glucose	≤14 0	8 1 (6)	7 9 (2)	10 6 (5)	3 4 (?)
	>14 0 ≤18 0	11 9 (?)	13 3 (5)	10 6 (4)	4 3 (3)
	>18 0	7 1 (2)	13 0 (5)	19 3 (1)	6 3 (5)
		9 0 (10)	11 3 (17)	13 3 (10)	4 6 (10)
		n s (<1 vs 1–4)	n s (1–4 vs 5–15)	<0 001 (5–15 vs adults)	
			<0 001 (1–4 vs adults)		
		<0 10 (<1 vs adults)			

Statistical calculations performed with multivariate analysis with weighted means (18) This method eliminates the effect of cell size (cell surface) on metabolism and permits analysis of the relations between age and metabolism

[a] Mean ±S D weighted means

[b] No further testing since $n=1$

hrs The basal lipolysis of the specimen from the newborn child was the highest in this group (112 6 nmoles/10[5] cells) while the specimen taken after 120 hrs was within the range of the other children (21 5 nmoles/10[5] cells)

Hormonal responsiveness

In contrast to glucagon (see below) noradrenalin consistently enhanced lipolysis in all age groups As shown in Table 1 the lipolytic rate was significantly higher in the child specimens than in the adults Due to the rather small biopsy samples obtained only one experiment with noradrenalin was performed with fat cells from a child below 1 yr of age (4 months) However this specimen responded briskly (Table 1) Although the lipolytic rates in response to noradrenalin were higher in the child groups the percentage increases over basal values were similar in both adults and children

Addition of glucagon at a high concentration did not elicit a consistent lipolytic effect in any child group (Table 2) Similarly no effect of glucagon on glucose metabolism was found in either child group (data not shown)

DISCUSSION

The present study was undertaken to analyse the effect of age on metabolism The data show that fat cell size is of importance for the

view of the findings discussed above it may well be then that fat cells from young individuals are reponsive to glucagon Since glucagon levels are elevated in newborns (20) it seems of importance to evaluate the possible physiological significance of this agent for lipid mobilization

In the present investigation, glucose incorporation and basal and stimulated lipolysis were evaluated in human adipose tissue with respect to both fat cell size and age

MATERIALS AND METHODS

Biopsies of abdominal subcutaneous adipose tissue were obtained from 32 children varying in age from a few hours up to 15 years The children were generally operated upon for inguinal hernia or retained testis Two newborns were included who were operated upon for esophagus atresia or an ovarian cyst The metabolic responses of fat cells from children were compared with the results obtained with abdominal subcutaneous fat from adults who were selected so as to have similar fat cells sizes as the children The adults were operated upon for cholecystolithiasis (1) gastric ulcer (2) varicocele (3) ovarian cyst (2) renal stones (1) or a hyperplastic prostate (1)

After excision smaller biopsies weighing about 20–30 mg each were prepared and preincubated for 30 min in the same medium as was later used for the incubations The biopsies were then placed in fresh medium and incubated for 2 hrs at 37 C The incubation medium used was medium 199 (Statens Bakteriologiska Laboratorium Stockholm) modified to a glucose concentration of 1 0 mM containing 40 g/l bovine albumin (Fraction V Sigma Chemical Co St Louis Mo) and the indicated concentrations of noradrenalin (noradrenalin bitartrate Astra AB Sodertalje Sweden) or glucagon (Novo A/S Copenhagen) In some experiments 0 15 μCi [1 ^{14}C]glucose (New England Nuclear Corp Frankfurt/Main W Germany) was added to the incubation medium and the incorporation into the triglycerides determined The release of glycerol to the incubation medium was determined enzymatically (19) and taken as an index of lipolysis

After the incubation period the tissue lipids were extracted with chloroform/methanol (2 1 v/v) as described by Folch et al (9) An aliquot of the chloroform phase was taken for the determination of the radioactivity in the triglycerides using a Packard Tri-Carb liquid scintillation spectrometer (Packard La Grange Illinois) Glyceride glycerol was determined on the chloroform phase as described by Carlson (6) After saponification the fatty acids were in some experiments extracted with *n* heptane and the radioactivity determined

The metabolic parameters studies were expressed in terms of the cellularity of the specimens as previously suggested (1 12 25) Fat cell size was determined after isolation with collagenase as previously described (27) The amount of collagenase used was the same for both children and adults 5 mg per 3 ml medium The error of the sizing procedure used has been found to be 3 4% (27) Mean cellular volume was calculated as described by Goldrick (10) and mean cellular weight according to Hirsch & Gallian (13) assuming that the density of fat cells equals that of triolein When the triglyceride content of the biopsies and the mean cellular weight are known the number of fat cells can be calculated

Since adipocyte size is an important parameter for metabolic rates (1 16 17 25 29) the measured parameters were related to fat cell surface area calculated from the measured diameters as described by Zinder & Shapiro (29)

RESULTS

The subjects were divided into four groups of equal size according to the age of the donors (below 1 yr 1–4 yrs 5–15 yrs and adults= 38 2±14 1 years mean ±S D)

Cell size

The correlations between basal lipolysis basal rate of glucose incorporation into triglycerides and cell surface area were significant for both adults and children between 5 and 15 years (r values between 0 581 and 0 828 $p<0$ 05) For the children below 1 year and between 1–4 years however the correlation coefficients did not reach statistical significance

Age

Since cell size influenced the rates of metabolism this factor had to be eliminated in order to analyse the effect of age This was performed by multivariate analysis with weighted means (18) As shown in Table 1 adults had lower rates of glucose incorporation and basal and stimulated lipolysis than the children The differences were highly significant for all parameters measured for children in the two oldest age groups For the youngest children however only a tendency to a difference ($p<0$ 10) was found for glucose incorporation

Between the three child groups there were no significant differences in the parameters measured except that basal lipolysis was lower in the youngest group In this group one specimen was obtained from a child within 24 hrs after birth and in another child after 120

Table 1 *Metabolic variables in relation to age*

Figures within parentheses denote number of donors

Variable studied (nmoles/10[5] cells)	Variable held constant cell surface ($n \times 10^{-3}$ μm^2)	Variable in relation to age (in years) <1	1–4	5–15	Adults[a] (38 2±14 1)
Basal glycerol release	≤14 0	37 5 (6)	59 7 (2)	49 2 (5)	17 3 (?)
	>14 0 ≤18 0	34 1 (?)	68 0 (5)	48 3 (4)	14 0 (3)
	>18 0	48 0 (2)	86 4 (5)	10? 4 (1)	22 4 (5)
		39 6 (10)	70 7 (1?)	65 4 (10)	18 1 (10)
		<0 05 (<1 vs 1–4)	n s (1–4 vs 5–15)		<0 01 (5–15 vs adults)
			n s (<1 vs 5–15)		
				<0 001 (1–4 vs adults)	
Glycerol release +5 0×10[-5] M noradrenalin	≤14 0	–	–	84 9 (2)	39 2 (?)
	>14 0 ≤18 0	–	147 5 (2)	73 3 (2)	29 7 (3)
	>18 0	165 8 (1)	179 6 (2)	149 7 (1)	38 0 (5)
		165 8 (1)[d]	138 0[c] (4)	134 0 (5)	34 1 (10)
			n s (1–4 vs 5–15)		
				<0 001 (1–4 vs adults)	
					<0 01 (5–15 vs adults)
Basal incorporation of glucose	≤14 0	8 1 (6)	7 9 (2)	10 6 (5)	3 4 (2)
	>14 0 ≤18 0	11 9 (2)	13 3 (5)	10 6 (4)	4 3 (3)
	>18 0	7 1 (?)	13 0 (5)	19 3 (1)	6 3 (5)
		9 0 (10)	11 3 (1?)	13 3 (10)	4 6 (10)
		n s (<1 vs 1–4)	n s (1–4 vs 5–15)		<0 001 (5–15 vs adults)
				<0 001 (1–4 vs adults)	
			<0 10 (<1 vs adults)		

Statistical calculations performed with multivariate analysis with weighted means (18) This method eliminates the effect of cell size (cell surface) on metabolism and permits analysis of the relations between age and metabolism

[a] Mean ±S D weighted means

[d] No further testing since $n = 1$

hrs The basal lipolysis of the specimen from the newborn child was the highest in this group (112 6 nmoles/10[5] cells) while the specimen taken after 120 hrs was within the range of the other children (21 5 nmoles/10[5] cells)

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Table 2 *Lipolysis in response to glucagon in fat cells from donors of varying age (expressed in nmoles/10⁵ cells)*[a]

Incubation conditions	Release of glycerol in age groups		
	<1 (n=3)	1–4 (n=4)	5–15 years (n=7)
Basal	35 7±22 2	93 6±8 2	68 9±10 6
Basal+ 5 0 µg/ml glucagon	38 6±26 2	89 3±1 5	70 9±12 7

[a] Results are means ±S E M

metabolic rates at least in older children and adults Since fat cell size varies with the degree of obesity (3) as well as the age of the donor (15, 23) this factor must be considered when the relationships between age and metabolism are evaluated In the present study this objective was reached in two ways Firstly the adults were selected so that their fat cell sizes were within the same range as the children Secondly in the statistical analyses the effects of fat cell size and age on metabolism were analysed independently The data show that the basal rates of lipolysis and glucose incorporation were higher in the children than in the adults Less pronounced differences were found for the children below one year of age, however Although we have very few data on metabolism in the immediate neonatal period the data support the findings of Novak et al (22) i e that the fat cells have an exceedingly high lipolytic rate in this period The main discrepancy between that study and the present one is our finding that the rate of lipolysis is considerably higher throughout childhood as compared with adults while Novak et al (22) found only a slight difference after the neonatal period This discrepancy may however be due entirely to the fact that possible differences in fat cell size were not accounted for in their study

Lipolysis in response to noradrenalin was higher in children than in adults when expressed as an absolute effect However on a percentage basis the increase was similar in all age groups It should be emphasized that in the present study a maximal concentration of noradrenalin was used Thus the increased absolute lipolytic effect means that the cellular *capacity is increased In order to evaluate* possible differences in cellular *sensitivity dose–response curves should be performed* (17) Due to the smallness of the tissue specimens usually obtained this was not possible in the present study

Of particular interest was the finding that glucagon failed to elicit a lipolytic response at any age It is well known that adult human adipose tissue responds only sluggishly (2) or not at all to this hormone (5) However in view of previous data showing that young rats are responsive to glucagon in contrast to old animals (14, 21) and that glucagon induced adenylate cyclase activity declines with age (7) an effect of glucagon on child adipose tissue could have been anticipated Since lipolysis seems particularly high immediately after birth as discussed above (24 28) and since glucagon levels are elevated after birth (20) such an effect would have had physiological implications From the present study however it seems clear that the increased lipolysis after birth is not due to a direct effect of glucagon on white fat cells The reason for this lack of effect of glucagon on human fat cells is not clear Since an effect of glucagon on glucose metabolism (known from other studies with rat fat cells (4)) was lacking as well this glucagon resistance may be due to a lack of membrane hormonal receptors

In analogy with the effect of age on lipolysis the rate of glucose conversion to triglycerides is also increased in the children Since adult human adipose tissue only synthesizes fatty acids to a very small extent (26) incorporation into total lipids was usually determined From studies on rat fat cells however it has been found that fatty acid synthesis decreases with increasing age (8) As shown in four control experiments about 20% of the total glu

cose uptake is recovered in the fatty acid moiety in child fat cells as compared with about 5% in adults (26) Thus the increased rate of glucose incorporation into the trigly cerides is due to an increase in the synthesis of both fatty acids and glyceride glycerol

The present evidence of increased basal lipolysis throughout childhood is somewhat at variance with a previous report (23) in which the authors using a tissue culture technique found that basal lipolysis was consistently higher only in fat cells from children below one year of age The reason for this discrepancy is presumably the difference in incubation tech niques used With the tissue culture technique the effects of host factors are presumably eli minated and a more basal state is reached than in the present short term incubations It may well be then that the increased meta bolic rates in child adipose tissue as compared with adults are primarily associated with exo genous factors such as differences in hormone levels (cf 11 20) and/or nutritional differences

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (B77 19X 3506) and the University of Gothenburg

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SCREENING FOR AUTOSOMAL ABERRATIONS

MAKOTO HIGURASHI[1] MASAYA SEGAWA[2] ICHIRO MATSUI[3]
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ABSTRACT Higurashi M Segawa M Matsui I Ihnuma K and Nakagome Y (Department of Maternal and Child Health and Department of Pediatrics University of Tokyo Tokyo Division of Medical Genetics Kanagawa Children s Medical Center Yokohama Department of Human Genetics National Institute of Genetics Mishima Japan) Screening for autosomal aberrations Acta Paediatr Scand 66 501 1977.—A method of screening for autosomal aberrations is important as an indication for chromosome analysis such as that used in sex-chromatin examination for sex chromosome aberrations In our clinic malformed patients with mental retardation and abnormal dermatoglyphic patterns are strong suspects for autosomal aberrations Abnormal dermatoglyphic patterns are separated into two categories (1) Absolutely abnormal—radial loop of 1st finger radial loop of 4th finger radial loop of 5th finger arch over 6 fingers arch tibial loop tibial and arch fibular (2) Borderline abnormalities—high axial triradius (t and t') simian crease interdigital loop and single crease of 5th finger Of 416 cases showing malformation retardation and abnormal dermatoglyphics 308 had autosomal aberrations while 108 had normal karyotypes In the group with autosomal aberrations 279 patients (90 6%) had absolutely abnormal dermatoglyphics In the group with normal karyotypes only 8 patients (7 4%) had absolutely normal dermatoglyphics while most had abnormal dermatoglyphics in the borderline category These clinical manifestations absolutely abnormal dermatoglyphics mental retardation and malformations are therefore very useful in screening for autosomal aberrations

KEY WORDS Screening autosomal aberrations dermatoglyphics

The rapid development of human cytogenetics during the past twenty years and the discovery of numerous human cytogenetic abnormalities have opened up new aspects of both human genetics and teratology (3 7 9) Cytogenetics laboratories may be asked to carry out chromosome investigations on individuals with congenital malformations but it is not yet feasible for all patients with malformations to be examined by chromosome analysis Some tools for the screening of autosomal aberrations if there were any such would be useful in the selection of indications for this examination such as sex-chromatin examinations as a screening method for sex chromosome aberrations (2 6)

Malformed patients with mental retardation and abnormal dermatoglyphic patterns attending our clinic have been suspected strongly of autosomal aberrations for these ten years All cases with the above Triad Congenital malformations mental retardation and abnormal dermatoglyphic patterns have undergone cytogenetic examinations and it has been found that these clinical manifestations are useful in screening for autosomal aberrations

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MATERIALS AND METHODS

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Submitted Sept 20 1976
Accepted Nov 17 1976

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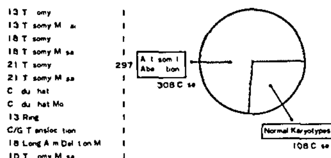


Fig 1 Materials

Clinic Department of Pediatrics University Hospital at the University of Tokyo The patients were of both sexes and all were younger than 15 years of age Abnormal dermatoglyphic patterns in Japanese as shown by Matsui et al (4) were separated into two categories (1) absolute abnormalities—radial loop of 5th finger radial loop of 4th finger radial loop of 1st finger arch over six fingers arch tibial loop tibial and arch fibular (2) borderline abnormalities—high axial triradius (t and t) simian crease interdigital loop and single crease of 5th finger Cytogenetic studies were usually done by analysis of cultured lymphocytes and of buccal smears for X and Y chromatin examinations

Patients with sex chromosome aberrations were not included in this study unless autosomal aberrations were also observed

RESULTS

(1) Cytogenetic observations are shown in Fig 1 Of 416 cases with multiple congenital malformations mental retardation and abnormal dermatoglyphic findings 308 cases had autosomal aberrations and 108 cases showed normal karyotypes No cases showed autosomal aberrations combined with some kind of sex chromosome abnormality

(2) The relationships between cytogenetic findings and dermatoglyphic findings are shown in Fig 2 In 416 cases with more than three clinical manifestations 308 cases had autosomal aberrations Some 279 cases out of 308 (90 6%) had completely abnormal dermatoglyphics In comparison only 8 cases out of 108 (7 4%) with normal karyotypes had completely abnormal dermatoglyphics Chromosome analyses for these 8 cases were examined further by binding techniques and most cases with normal karyotypes had abnormal dermatoglyphics of the borderline category Since some cases had more than one abnormal finding the total number of abnormalities shown is greater than the number of cases examined

(3) Sex ratio maternal age at birth history of abortions or stillbirth in the patients mothers and gestational age at birth were all compared in cases of autosomal aberrations and normal karyotypes (Table 1 and Fig 3) The data for controls in Table 1 were obtained from the source by the Ministry of Health and Welfare in Japan (5) In both groups there were more males than females The maternal age at birth was significantly higher for cases with autosomal aberrations than for controls The incidence of abortion and stillbirth was greater in the cases with autosomal aberration than in the cases with normal karyotypes There was no significant difference in gestational age at birth in the two groups

DISCUSSION

Cytogenetic techniques have played a major role in elucidating factors responsible for developmental abnormalities of the human reproductive system Many syndromes with chromosome abnormalities have been identified from congenital malformations and established as disease entities On the other hand many congenitally malformed patients have been subjected to cytogenetic examination and yet have shown no chromosome aberrations

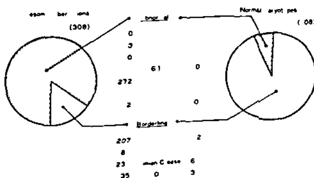


Fig 2 Incidence of abnormal findings in dermatoglyphics

Table 1 *Comparison between autosomal aberrations and normal karyotypes*

	Autosomal aberrations	Normal karyotypes	Controls
Sex ratio (male/female)	1 139	1 51?	1 035–1 06
Maternal age at birth			
–19 years	0	0	0 010
?0–?9 years	0 508	0 680	0 757
30–34 years	0 ?87	0 64	0 185
35–39 years	0 163	0 04?	0 04?
40– years	0 04?	0 014	0 006
History of abortion or stillbirth			
1×	0 163	0 080	
?×	0 051	0 010	
3×	0 030	0 031	
4× or over	0 007	0	

Therefore the complex and tedious nature of chromosome analysis cannot be justified in all cases of congenital malformation A method of screening for autosomal aberrations is of importance prior to chromosome analysis

Screening for sex chromosome aberrations is carried out using the sex chromatin examination where it is possible to examine many samples within a short time For several years we have been trying to characterize the common features in autosomal aberrations in order to establish useful screening methods Consequently mental retardation congenital multiple malformations abnormal dermatoglyphics (1 8 10) small for date babies and failure to thrive have been found to be fundamentally common features in almost all auto-

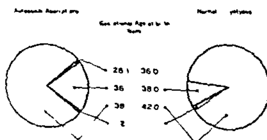


Fig 3 Comparison between autosomal aberrations and normal karyotype

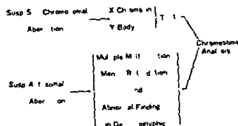


Fig 4 Screening method for chromosomal aberrations

somal aberrations provided that cases of reciprocal translocation were excluded The first three clinical features named were found to be especially common

On the basis of the findings 416 patients with these three clinical features who attended our out patient clinic were selected at random In 416 patients with the above three clinical features 74 03% had autosomal aberrations Of these cases 90 6% had absolutely abnormal dermatoglyphics when borderline cases were excluded However we consider that the cases with abnormal dermatoglyphics in the borderline category should not be excluded from screening of autosomal aberrations i e all abnormal findings in dermatoglyphics should be included for screening because small but significant numbers of patients with autosomal aberrations have abnormal dermatoglyphic patterns in the borderline category

Maternal age at birth and history of abortion or stillbirth may be supplementary tools for screening although they are not always useful

As a result of our findings we have established screening methods for chromosome aberrations which are routinely carried out in our clinic and these are summarized in Fig 4

ACKNOWLEDGEMENT

The authors wish to express their thanks to Dr J Sturgess the Department of Pathology Research Institute Hospital for Sick Children Toronto Canada for her advice on preparation of this paper including revision of the English

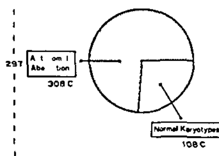


Fig 1 Materials

Clinic Department of Pediatrics University Hospital at the University of Tokyo The patients were of both sexes and all were younger than 15 years of age Abnormal dermatoglyphic patterns in Japanese as shown by Matsui et al (4) were separated into two categories (1) absolute abnormalities—radial loop of 5th finger radial loop of 4th finger radial loop of 1st finger arch over six fingers arch tibial loop tibial and arch fibular (2) borderline abnormalities—high axial triradius (t and t″) simian crease interdigital loop and single crease of 5th finger Cytogenetic studies were usually done by analysis of cultured lymphocytes and of buccal smears for X and Y chromatin examinations

Patients with sex chromosome aberrations were not included in this study unless autosomal aberrations were also observed

RESULTS

(1) Cytogenetic observations are shown in Fig 1 Of 416 cases with multiple congenital malformations mental retardation and abnormal dermatoglyphic findings 308 cases had autosomal aberrations and 108 cases showed normal karyotypes No cases showed autosomal aberrations combined with some kind of sex chromosome abnormality

(2) The relationships between cytogenetic findings and dermatoglyphic findings are shown in Fig 2 In 416 cases with more than three clinical manifestations 308 cases had autosomal aberrations Some 279 cases out of 308 (90 6%) had completely abnormal dermatoglyphics In comparison, only 8 cases out of 108 (7 4%) with normal karyotypes had completely abnormal dermatoglyphics Chromosome analyses for these 8 cases were examined further by binding techniques and most cases with normal karyotypes had abnormal dermatoglyphics of the borderline category Since some cases had more than one abnormal finding the total number of abnormalities shown is greater than the number of cases examined

(3) Sex ratio maternal age at birth history of abortions or stillbirth in the patients mothers and gestational age at birth were all compared in cases of autosomal aberrations and normal karyotypes (Table 1 and Fig 3) The data for controls in Table 1 were obtained from the source by the Ministry of Health and Welfare in Japan (5) In both groups there were more males than females The maternal age at birth was significantly higher for cases with autosomal aberrations than for controls The incidence of abortion and stillbirth was greater in the cases with autosomal aberration than in the cases with normal karyotypes There was no significant difference in gestational age at birth in the two groups

DISCUSSION

Cytogenetic techniques have played a major role in elucidating factors responsible for developmental abnormalities of the human reproductive system Many syndromes with chromosome abnormalities have been identified from congenital malformations and established as disease entities On the other hand many congenitally malformed patients have been subjected to cytogenetic examination and yet have shown no chromosome aberrations

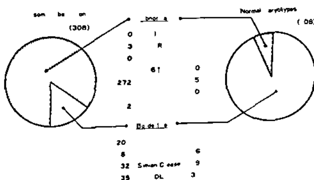


Fig 2 Incidence of abnormal findings in dermatoglyphics

Acta Pædiatr Scand 66 505–511 1977

FACTORS RELATED TO EARLY TERMINATION OF BREAST FEEDING

A Retrospective Study in Sweden

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ABSTRACT Sjolin S Hofvander Y and Hillervik C (Department of Paediatrics University Hospital Uppsala and Institute of Sociology Uppsala University Uppsala Sweden) Factors related to early termination of breast feeding A retrospective study in Sweden Acta Paediatr Scand 66 505 1977 —In 1972 298 mothers representative of the city of Uppsala in general breast fed their babies for only a brief period of time despite a clear wish to continue—36% up to 8 weeks The most common reason for terminating breast feeding was that the milk dried up (66%) More precise reasons commonly mentioned were anxiety of all kinds lack of motivation stress tiredness and work outside the home It was further found that mothers who enjoyed their breast feeding were well educated were older than 25 years and belonged to social class 1 tended to breast feed longest

KEY WORDS Breast feeding

During the last few decades breast feeding has been continuously declining in practically all industrialized countries In the United States this trend has been obvious for a fairly long time and in 1966 only about 18% of the mothers breast fed their babies when leaving the maternity hospitals (2) Reports indicate that the situation is basically the same in Europe though in some countries particularly in Eastern Europe the breast feeding rate is higher (cf 8)

A downward trend has also been observed in recent years in developing countries especially in urban areas This is a subject of considerable concern as a short breast feeding period with early introduction of breast milk substitutes will often lead to serious intestinal infection and malnutrition in these countries (4)

In Sweden there has been a continuous decline in the breast feeding rate over the last 40 years In 1944 56% of the infants were being breast fed at 6 months while in 1970 this figure was only 7% (7)

The reasons for this dramatic change are only partly known Presumably there are a number of underlying factors which are interrelated vary between different individuals and countries and are associated with the social and economic development at large Only few studies however have been undertaken with the aim of analysing the influence of such factors (1 3 6)

The present paper reports a Swedish study concerning the duration of breast feeding the mothers attitudes towards it and reasons for early weaning

The study was supported by grants from the Swedish Nutrition Foundation and the Stockholm Child Welfare Committee

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Submitted Sept 20 1976
Accepted Nov 28 1976

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Table 3 *Reasons stated by mothers for never having started breast feeding*

	No
Low birth weight	13
Mother ill	8
Previous unpleasant experience	4
No milk	2
No clear explanation	4
N=31 (10 4% of 298)	

Table 4 *Reasons stated by mothers for terminating breast feeding before 6 months*

	No	%	
Sore nipple milk congestion	24	9 4	14 5
Child ill	13	5 1	
Milk dried up	169	66 3	85 5
Other reason	49	19 2	
N=255 (85 6% of 298)		100 0	

RESULTS

A *Duration of breast feeding*

The durations of partial and complete breast feeding are shown in Table 2 The most rapid decline took place during the first month On discharge from the maternity ward usually on the fifth or sixth day 77 8% of the 298 mothers were breast feeding completely and 11 8% partially At 6 months these figures were only 1 0% and 3 0% respectively

B *Reasons for terminating breast feeding*

Of all the 298 mothers 31 (10 4%) had never breast fed at all The reasons stated are given in Table 3 As Table 2 shows 12 mothers (4%) were still breast feeding when the study was finished at 6 months

All the other 255 mothers had started breast feeding but stopped before 6 months Their reasons for termination are seen in Table 4 Only in 14 5% could the reason be clearly and immediately identified The 218 mothers who gave the answer milk dried up or other reason were immediately asked to try to give a more precise answer Thirty one of them were not able to give any further information The more specified reasons for the others are seen in Table 5 The great variety of reasons is striking Anxiety of different kinds stress tiredness lack of motivation and occupations away from the home seemed to be the most important determinants

All the mothers participating in the study (298) were also asked to give their opinion on why other mothers did not want to continue breast feeding for as long as 6 months Some mothers gave several reasons The results are shown in Table 5 together with the reasons for their own discontinuation It seemed to be the general opinion that other mothers had to face the same kind of problems that they had experienced themselves Some exceptions however were noted The interviewees for example considered the reasons wet and messy inconvenience and concern for figure and breast shape to be much more common for others than for themselves

Table 5 *Specified reasons for termination of breast feeding given by mothers who weaned before 6 months (For self) and reasons suggested by all 298 mothers for termination by others (For others)*

	For self N=187 (%)	For others" N=298 (%)
Doubted that the child got sufficient weight control difficult mixed feeding difficult	16 6	22 5
Anxiety insecurity	9 6	3 4
Lack of motivation	9 1	7 7
Stress	9 1	5 4
Studies employment outside the home	7 5	6 7
Wet messy	6 4	26 2
Inconvenience	6 4	38 9
Mental tiredness	6 4	7 0
Physical tiredness	5 9	10 1
Other children not time to rest	5 3	3 7
Dislike of breast feeding	1 1	5 0
Concern for figure and breast shape	0 5	12 1
Breast feeding painful	0 0	3 0
Other reason	16 1	10 1
	100 0	161 8

MATERIAL AND METHODS

The study was conducted in the city of Uppsala (130 000 inhabitants) in April–June 1972 as a standardized interview lasting about one hour The ages of the children varied between 6 and 13 months at the time of the interview The interviews were conducted by one of the authors (C H) and by three students from the Institute of Sociology—the interviewers working in close collaboration with us and with the Child Health Centres

The original random sample consisted of 320 mothers or exactly 20% of all women living in Uppsala who had given birth to a baby in 1971 The final sample was reduced to 298 mothers (93 1% of the original sample) Ten (3 1%) refused to take part in the study and the remaining 12 (3 8%) had moved and could not be easily traced All mothers were delivered at the University Hospital

The age distribution of the mothers is seen in Table 1 The majority were married (Table 7) Of the mothers 58% were primiparae and 31% had two children

From the interview information was obtained concerning

- the duration of breast feeding (A under Results)
- the reasons for terminating breast feeding (B under Results)
- the mother s attitude to breast feeding (C under Results)

A number of factors that were presumed to be related to breast feeding such as the mother s age civil status occupation education social class etc were also recorded

With the aim of revealing reasons for early termination of breast feeding the relation between the duration of breast feeding and a series of other factors was analysed (D and E under Results) For this analysis the material was systematically divided into two groups—one consisting of all mothers who breast fed for less than 8 weeks and the other of those who breast fed for 8 weeks or more The attitudes towards breast feeding and the reasons for early weaning were also examined in different subgroups of mothers (F and G under Results)

Statistical methods

We have a number of 2×2 contingency tables in which we compare two different groups (I and II) concerning some property (+ or −)

	I	II	
+	A	B	A+B
−	C	D	C+D
	A+C	B+D	A+B+C+D=N

Now we want to test whether the groups differ concerning this property The technique is as follows 1) State a null hypothesis (H_0) There is no difference between the two groups 2) State an alternative hypothesis (H_1) One of the groups tends to show a greater frequency for some property 3) We test this by computing the probability of the given or more extreme values under the assumption of H_0 4) If we obtain a low probability (below 5%) we reject H_0 in favour of H_1 This denotes a significant result at the 5% level

A method which computes this is the Fisher exact probability test but it has the disadvantage of lending itself to tiresome computations Besides it has the restriction of regarding the margin sums as constant Other methods are therefore often used among which the χ^2 test is the most common In the present study the following formula (5) was used

$$\chi^2 = \frac{N\left(|AD-BC|-\frac{N}{2}\right)^2}{(A+B)(C+D)(A+C)(B+D)}$$

The formula gives the χ^2 value with one degree of freedom From a table the corresponding value of the distribution function can be obtained which is the same as the probability described above (in reality its complement) The test assumes independent groups The formula incorporates a correction for continuity which improves the approximation In practice we have a variable that only gives certain values while χ^2 is a continuous variable The question is When is the approximation good? As a rule of thumb Siegel gives $N>40$ This requirement is met by the data in all our tables but in some of them the frequency of an individual group is small making the conclusions more uncertain in those cases

Table 1 *Age of the mothers at delivery*

Age (y)	%
15–19	5 7
20–24	31 8
25–29	47 7
≥30	14 8
N=298	100 0

Table 2 *Duration of breast feeding*

Time in weeks	Wholly breast fed (%)	Partially breast fed (%)	Wholly and partially breast fed (%)	Not breast fed (%)
0	77 8	11 8	89 6	10 4
2	65 5	17 8	83 3	16 7
4	39 5	18 5	58 1	41 9
8	22 1	14 5	36 6	63 4
12	12 5	7 0	19 5	80 5
16	6 0	7 8	13 8	86 2
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At discharge from the maternity ward

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16	6 0	7 8	13 8	86 2
20	1 6	7 0	8 6	91 4
24	1 0	3 0	4 0	96 0
N=298				

At discharge from the maternity ward

Table 9 *Relation between the mother s social class and the duration of breast feeding (per centage number in each social group)*

Social class 1 vs 3 χ^2=3 93 significant difference at the 5% level Otherwise no significant differences between the different social classes were found

Duration of breast feeding in weeks	Social class 1 (%)	2 (%)	3 (%)
<8	51 4	57 1	67 1
≥8	48 6	42 9	3[illegible] 9
N	109	70	70

longer than in the other five and in two dis tricts the breast feeding period tended to be strikingly short No definite explanation was found for these differences

E *Factors probably not related to the duration of breast feeding*

Neither parity the child s sex the duration of the child s stay in the maternity ward the time of the first coition after delivery nor the mothers political sympathies seemed to influ ence the duration of breast feeding No mother had started to take contraceptive pills before the termination of breast feeding

F *Reasons for terminating breast feeding in different groups of mothers*

Mothers who had left school relatively early more often terminated breast feeding because of anxiety than those with a higher educa tional background (>10 years) The difference was significant at the 1% level Otherwise no significant correlations were found between different groups of mothers and special rea sons for weaning Some tendencies however may be worth reporting

The younger mothers tended to give anx iety as a reason more often than did the older ones The unmarried mothers who were cohabiting with the child s father most com monly stated inconvenience as the reasons Students most often mentioned stress or mental tiredness and housewives mental tiredness as their reason for the termination In social class 1 employment away from the home studies mental tiredness and stress were the most common reasons while in social class 3 lack of motivation anxiety and technical difficulties were most frequent

G *Attitudes to breast feeding in different groups of mothers*

Different groups of mothers as defined under D and E did not hold significantly divergent attitudes to breast feeding immediately after delivery or later

CONCLUSIONS AND DISCUSSION

It should be emphasized that the mothers par ticipating in this study were not representative of all Swedish towns or of the rural areas In particular there was an overrepresentation of mothers from social class 1—40 5% in compa rison with 10 5% for the country as a whole Furthermore it is evident that mothers with a higher education and students were more numerous in this material than are found on an average in Sweden The number of mothers of social classes 2 and 3 however was suffi ciently large to permit statistical analyses of all subgroups of interest In conclusion the re sults may be safely regarded as representa tive of a typical Swedish university city but at the same time there is every reason to con

Table 10 *Relation between the mother's ex perience of breast feeding and the duration of breast feeding (percentage number in each ex perience group)*

Positive experience vs not positive χ^2 11 0 significant difference at the 0 1% level

Duration of breast feeding in weeks	Experience of breast feeding: Positive (%)	Not positive (%)
<8	53 0	76 8
≥8	47 0	23 [illegible]
N	198	69

Table 6 *Relation between the mother's age and the duration of breast feeding (percentage number in each age group)*

<25 vs ≥25 years $\chi^2=9.91$ denoting a significant difference at the 0 5% level

Duration of breast feeding in weeks	Age (y)	
	<25 (%)	≥25 (%)
<8	72 7	52 3
≥8	27 2	47 6
N	99	168

Table 7 *Relation between the mothers civil status and the duration of breast feeding (percentage number in each group)*

Married+not married cohabiting vs single $\chi^2=3.4?$ not significant

Duration of breast feeding in weeks	Married (%)	Not married cohabiting (%)	Single (%)
<8	57 9	59 0	83 3
≥8	42 1	41 0	16 7
N	216	39	1?

C *The mothers attitudes to breast feeding*

Immediately after delivery 91 6% of all the mothers had expressed a clear wish to breast feed, 60% of them for as long as possible Only 8 4% had for various reasons decided not to breast feed The great majority (74 6%) regardless of their original attitude stated later that they had enjoyed breast feeding their babies Only 12 4% frankly admitted that they genuinely disliked it while the rest of the mothers seemed to be more or less neutral

D *Factors related to the duration of breast feeding*

Age Table 6 shows that mothers who were 25 years of age or older tended to breast feed longer than the younger mothers The difference was significant

Civil status In Table 7 all mothers who cohabited with the child s father are compared with all single mothers There was no significant difference between the two groups in relation to duration of breast feeding but it should be noted that the number of single mothers was only 12

Occupation Students more often breast fed for 8 weeks or more than did housewives but there was no significant difference between the two groups

Education From Table 8 it is seen that mothers with higher education tended to breast feed longer than those with less schooling The difference was not significant between 6–7 and 9–10 school years but between all the other groups taken separately or combined, the differences were significant

Social class Mothers of social class 1 tended to breast feed longer than those of class 2 who in turn tended to continue longer than mothers of class 3 (Table 9) However, the only significant difference was obtained between social classes 1 and 3

Mothers experience of breast feeding The mothers who had experienced breast feeding as something positive breast fed their infants significantly longer than those whose experience was not positive (Table 10)

Child Health Districts In two districts out of seven the mothers tended to breast feed

Table 8 *Relation between the mother s education and the duration of breast feeding (percentage number in each educational group)*

Duration of breast feeding in weeks	Duration of education in years		
	6–7 (%)	9–10 (%)	>10 (%)
<8	72 2	62 4	47 1
≥8	27 8	37 6	52 9
N	72	93	102

6–7 vs >10 years $\chi^2=9.93$ significant difference at the 0 5% level
6–7+9–10 vs >10 years $\chi^2=9.24$ significant difference at the 0 5% level
6–7 vs 9–10+>10 years $\chi^2=6.23$ significant difference at the 2 5% level
9–10 vs >10 years $\chi^2=4.00$ significant difference at the 5% level
6–7 vs 9–10 years $\chi^2=1.35$ not significant difference

It was concluded that the true reasons for early weaning could not be revealed merely by an epidemiological retrospective approach Therefore a prospective detailed and intensive study of the course of breast feeding in individual women seemed greatly needed and is now in progress

ACKNOWLEDGEMENT

We would like to express our gratitude to Agneta Karlqvist B Sc Margareta Krantz B Sc and Susanne Bering B Sc for skilfully conducting part of the interviews We are greatly indebted to Gosta Hagglund B Sc for his expert help with the statistical analyses

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Submitted Oct 1 1976
Accepted Febr 5 1977

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sider many of the results as being of more general interest and applicable to other settings especially in the Western part of the world

Through this study some fundamental facts came to light which seemed to be more or less closely related to the mothers ability to breast feed their babies for an adequate length of time In general mothers who breast fed longest

- enjoyed breast feeding
- were well educated
- were older than 25 years
- belonged to social class 1
- were registered at one of two particular Child Health Centres
- were married or cohabiting with the child s father

The reasons given for termination of breast feeding offer further information as to the problem of early weaning The most common reasons given were

- anxiety for the child
- anxiety of other kinds
- lack of motivation
- stress and mental tiredness
- employment outside the home studies
- *inconvenience*

It was surprising to find that so many mothers were not able to point out the direct reason for weaning Most mothers were of the firm opinion that the milk simply had dried up Only after further questioning did most of them but not all give a reason that seemed more likely to be the true one though we were not convinced in all cases that this was the actual underlying factor This obviously means that the reasons given in Table 5 are far from proven There is also indirect evidence (Table 5) that many mothers regarded breast feeding as more messy and inconvenient and more damaging to the figure than they dared to admit to themselves let alone to the interviewer

It is worthy of observation further that although so many mothers wanted to breast feed and also enjoyed it very few were successful This probably means that one or several positive environmental factors were lacking and/or that some negative factors disturbed and spoiled the normal course of breast feeding

It seems that mothers who are mature experienced well educated and live under stable and good socio economic conditions are in a better position to go on with breast feeding than those who are less fortunate in these respects It would seem that mothers who are able to obtain evaluate and use information correctly are more likely to breast feed successfully These mothers probably also become less easily confused by the widespread seemingly or actually contradictive or biased advice issuing from mass media health personnel relatives friends and advertisements

The fact that anxiety was given as an important reason for termination of breast feeding by the young mothers and by the least educated probably reflects a common situation in an industrialized society of today It also points to the group of mothers to whom support and intensified information about breast feeding should be primarily directed

The findings in and conclusions drawn from this investigation are in essential agreement with the results of other recent studies (1 3)

Although this study has disclosed many factors which help to explain the reasons for early weaning it has become more and more obvious to us that other reasons may very well be of more immediate importance These factors would seem to be connected directly with the individual mother and her immediate environment with her personality her emotions her relations to husband and family and with her response to all kinds of minor everyday problems There is also reason to suspect that a mother s failure to continue breast feeding despite a strong wish is often due to a lack of access to prompt and adequate support from Child Health Centres or experienced persons from within or outside the family

It was concluded that the true reasons for early weaning could not be revealed merely by an epidemiological retrospective approach Therefore a prospective detailed and intensive study of the course of breast feeding in individual women seemed greatly needed and is now in progress

ACKNOWLEDGEMENT

We would like to express our gratitude to Agneta Karlqvist B Sc Margareta Krantz B Sc and Susanne Bening B Sc for skilfully conducting part of the interviews We are greatly indebted to Costa Hagglund B Sc for his expert help with the statistical analyses

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Submitted Oct 1 1976
Accepted Febr 5 1977

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sider many of the results as being of more general interest and applicable to other settings, especially in the Western part of the world

Through this study some fundamental facts came to light which seemed to be more or less closely related to the mothers ability to breast feed their babies for an adequate length of time In general mothers who breast fed longest

- enjoyed breast feeding
- were well educated
- were older than 25 years
- belonged to social class 1
- were registered at one of two particular Child Health Centres
- were married or cohabiting with the child s father

The reasons given for termination of breast feeding offer further information as to the problem of early weaning The most common reasons given were

- anxiety for the child
- anxiety of other kinds
- lack of motivation
- stress and mental tiredness
- employment outside the home studies
- *inconvenience*

It was surprising to find that so many mothers were not able to point out the direct reason for weaning Most mothers were of the firm opinion that the milk simply had dried up Only after further questioning did most of them but not all give a reason that seemed more likely to be the true one though we were not convinced in all cases that this was the actual underlying factor This obviously means that the reasons given in Table 5 are far from proven There is also indirect evidence (Table 5) that many mothers regarded breast feeding as more messy and inconvenient and more damaging to the figure than they dared to admit to themselves let alone to the interviewer

It is worthy of observation, further that although so many mothers wanted to breast feed and also enjoyed it very few were successful This probably means that one or several positive environmental factors were lacking and/or that some negative factors disturbed and spoiled the normal course of breast feeding

It seems that mothers who are mature experienced well educated and live under stable and good socio economic conditions are in a better position to go on with breast feeding than those who are less fortunate in these respects It would seem that mothers who are able to obtain evaluate and use information correctly are more likely to breast feed successfully These mothers probably also become less easily confused by the widespread seemingly or actually contradictive or biased advice issuing from mass media health personnel relatives friends and advertisements

The fact that anxiety was given as an important reason for termination of breast feeding by the young mothers and by the least educated probably reflects a common situation in an industrialized society of today It also points to the group of mothers to whom support and intensified information about breast feeding should be primarily directed

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Acta Pædiatr Scand 66 513–517 1977

AN EPIDEMIOLOGICAL STUDY OF HAEMOGLOBIN LEVELS IN INFANCY IN JERUSALEM

The Effect of Social Factors and the Relation to Physical Growth

H PALTI B ADLER and N WOLF

From the Department of Social Medicine The Hebrew University Hadassah Medical School Jerusalem Israel

ABSTRACT Palti H Adler B and Wolf N (Department of Social Medicine the Hebrew University Hadassah Medical School Jerusalem Israel) An epidemiological study of haemoglobin levels in infancy in Jerusalem The effect of social factors and the relation to physical growth Acta Paediatr Scand 66 513 1977.—The infant population of a mixed middle and low social class community in a western neighbourhood of Jerusalem has been examined for haemoglobin haematocrit weight and length at 9 months of age The mean haemoglobin has been 11 1 g/100 ml the mean haematocrit 36 1 and the mean MCHC 31 1% In most cases the anemia has been mild Statistically significant differences in the mean haemoglobin have been found by social class of father and sex of the child No statistically significant association has been found between birth weight weight and length at 9 months and mean weight increment per week between birth and 9 months of age and haemoglobin Lower levels of Hb have been demonstrated in the males by categories of birth weight and categories of weight increment

KEY WORDS Haemoglobin social factors physical growth sex

Anemia due to iron deficiency has been shown to be prevalent in early childhood in Israel (6 8 12)

A surveillance program of the physical and psychomotor development of 0–5 year old children has been established in a neighbourhood of Jerusalem (4) All babies of the community have also haemoglobin and haematocrit examinations carried out at 9 months of age The aim of this being to obtain information about the distribution of haemoglobin and to screen for anemia needing treatment

The haematological and growth data allow for study of the correlation between haemoglobin and physical growth

MATERIAL

Kiryat Hayovel is a suburb of the city of Jerusalem which is 800 meters above sea level The population of Kiryat Hayovel is about 15000 The social class distribution of the babies in this study population (n=858) by fathers occupation indicates that 19% are social class I+II (the upper classes) 31% social class III$_1$ (white collar) 33% social class III$_2$ (blue collar) 10% social class IV & V (lower classes) and 7% students The social class scale is an adaptation of the British Registrar Generals Occupational Rating (5)

Babies of families living in Kiryat Hayovel and born between June 1st 1971 and December 31st 1973 have been included in this study 9 % of those living in the area at 9 months were examined and blood samples collected (n=858) at the neighbourhood health center of the Hadassah-Hebrew University Department of Social Medicine

The study of weight and length was confined to those born between June 1st 1971 and May 31st 1972 (n=465) Low birth weight babies were excluded from the analysis

METHODS

The babies were examined between 9 and 10 months of age the clinical and developmental examination being performed by a physician and anthropometry by specially trained nurses with standard equipment (3) Stuart s weight and length percentiles were interpolated into 5 percentile categories for weekly age intervals by sex On the

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Table 2 *Mean haemoglobin at 9 month by birth weight and sex*

Birth weight (g)	Mean haemoglobin				
	Male	Female	Total	S D	*n*
2 501 2 750	10 1	11 2	10 7	1 5	57
2 751–3 000	10 7	11 2	11 0	1 1	109
3 001–3 250	10 9	11 3	11 2	1 2	149
3 251 3 500	10 8	11 1	11 0	1 0	161
3 501–3 750	10 9	11 4	11 1	1 2	80
3 751–4 000	11 0	11 5	11 2	1 3	34
4 000+	11 4	11 3	11 3	1 2	29
Total	10 7	11 2	11 0	1 2	–
n	304	310	–	–	614

($p<0\ 001$) see text

rank and sex of child on the haemoglobin level as calculated by analysis of variance is shown in Table 1

The mean haemoglobin level of males was 10 96 g/100 ml and than that of females 11 30 g/100 ml ($p=0\ 001$)

The difference in haemoglobin by social class of father was also found to be significant ($p=0\ 014$) Children born to fathers from social class I+II had the highest mean level of haemoglobin 11 44 g/100 ml No significant differences were found in mean haemoglobin by region of birth or education of mother and by birth rank

The five variables included in the analysis explain 6% of the variance

(c) *Relation between physical measurements and haemoglobin*

Birth weight The mean birthweight for males was 3 330 g S D 406 g and for females 3 240 g S D 317 The difference in birthweight by sex was statistically significant ($p=0\ 002$) Lower mean haemoglobin are noticed in the 2 501–3 000 g birth weight group than in the other groups This difference is not statistically significant neither for males nor for females (Table 2)

The haemoglobin level of males was consistently lower than that of females the difference being statistically significant when analysed according to birth weight categories ($p<0\ 001$)

The percentile for weight and length at 9 months of age was calculated for each sex separately No statistically significant differences were found in the mean haemoglobin by the percentile of weight or length at 9 month of age in the group as a whole and in each sex separately The difference in haemoglobin was statistically not significant by length

Mean increment in weight per week was calculated Those who had a mean increment of 130–150 g/week had the highest mean haemoglobin at 9 month of age both males and females The differences between the categories of increment in each sex separately and in the total population were not significant but in the categories of weight increments the difference between the sexes was significant ($p<0\ 003$) (Table 3)

DISCUSSION

The present study provides information about the infant population of a mixed middle and lower social class community in a western neighbourhood of Jerusalem at the age of 9 months The mean haemoglobin of 11 1 g/100 ml found in this population is somewhat lower than that found by Burman in Bristol (2) at the same age or by Samuelson (9) in North Sweden at 1 year of age

The distribution of the haemoglobin indi

Table 3 *Mean haemoglobin by mean increment per week between birth and 9 month of age by sex*

Weight increment (g/week)	Mean haemoglobin g/100 ml					
	Male	*n*	Female	*n*	Total	*n*
80–109	10 5	18	11 2	51	11 0	69
110–129	10 7	47	11 3	77	11 0	124
130–159	11 0	119	11 4	93	11 8	222
160+	10 9	45	10 8	33	10 9	59
Total	10 9	230	11 3	234	11 1	464

($p<0\ 003$) see text.

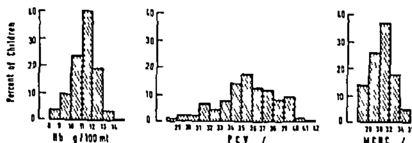


Fig 1 Distribution of Hb PCV and MCHC of 9 month old infants (n=858)

basis of these tables weight and length measurements were translated into percentiles by computor Weight increment was calculated from birth to the date of measurement and from it the average increment per week was computed

Haemoglobin and haematocrit determinations were done by special appointment none during acute febrile illnesses Capillary blood samples were taken from finger prick without compression of the finger Haemoglobin concentration was determined as cyanmethaemoglobin Interobserver variation was checked in 135 cases by two independent observers doing the determination twice on the same blood sample The mean of the difference was 0 11 g/100 ml The error as calculated by Samuelson (9) was found to be $\pm$0 27 g/100 ml

Packed cell volume was determined with a microhaematocrit centerifuge

Statistical analysis Means and standard deviations for haemoglobin and haematocrit were calculated The effect of 5 factors namely sex birth rank social class mothers educational standard and region of origin was determined by analysis of variance (10) The effect of one factor on the haemoglobin was calculated while controlling for the other variables included in the analysis The percent of the explained variance due to the above mentioned variables was calculated

The one way analysis of variance was used to study the effect of growth on the haemoglobin in each sex separately To study the sex differences in haemoglobin while controlling for the growth variable the Z test was used Both techniques were used in Tables 2 and 3

RESULTS

(*a*) *Haematological results*

The distribution of haemoglobin PCV and MCHC for the whole community of children is presented in Fig 1

Haemoglobin The mean haemoglobin is 11 1 g/100 ml S D 1 2 (n=858) A haemoglobin level below 9 g/100 ml was found in 3 8% of children below 10 g/100 ml in 13 5% and below 11 g/100 ml in 37 3%

Packed cell volume The mean PCV is 36 1 S D 2 8 (n=845) A haematocrit level below 34 was found in 16 8% of children

Mean corpuscular haemoglobin concentration The mean MCHC was 31 1% S D 2 5 An MCHC less than 31 was found in 40% of children

(*b*) *Effect of sociocultural background on the haemoglobin level*

The separate effect of social class of father education and region of origin of mother birth

Table 1 *The effect of social class of father education and region of origin of mother birth rank and sex of child on the haemoglobin at 9 month of age*

	Haemoglobin g/100 ml			
	n	Effect of factor	Mean	
Social class of father				
I+II	158	+0 31	11 44	p=0 014
III$_1$	264	−0 02	11 11	
III$_2$	276	−0 12	11 01	
IV–V	85	−0 21	10 92	
Students	59	+0 12	11 25	
Education of mother				
0– 4	29	−0 10	11 03	N S
5– 8	172	+0 04	11 17	
9–12	418	+0 03	11 16	
13+	223	−0 01	11 12	
Region of birth				
Israel	360	−0 02	11 11	N S
Asia	117	−0 06	11 07	
N Africa	246	−0 01	11 12	
Eur /Amer	119	+0 13	11 [illegible]6	
Birth rank				
1	353	+0 07	11 20	N S
2	66	−0 14	10 94	
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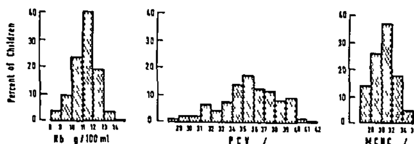


Fig 1 Dıstrıbutıon of Hb PCV and MCHC of 9 month old ınfants (*n*=858)

basıs of these tables weıght and length measurements were translated ınto percentıles by computor Weıght ın crement was calculated from bırth to the date of measure ment and from ıt the average ıncrement per week was computed

Haemoglobın and haematocrıt determınatıons were done by specıal appoıntment none durıng acute febrıle ıll nesses Capıllary blood samples were taken from finger prıck wıthout compressıon of the finger Haemoglobın concentratıon was determıned as cyanmethaemoglobın Interobserver varıatıon was checked ın 135 cases by two ındependent observers doıng the determınatıon twıce on the same blood sample The mean of the dıfference was 0 11 g/100 ml The error as calculated by Samuelson (9) was found to be ±0 27 g/100 ml

Packed cell volume was determıned wıth a mıcrohaema tocrıt centerıfuge

Statıstıcal analysıs Means and standard devıatıons for haemoglobın and haematocrıt were calculated The effect of 5 factors namely sex bırth rank socıal class mothers educatıonal standard and regıon of orıgın was determıned by analysıs of varıance (10) The effect of one factor on the haemoglobın was calculated whıle controllıng for the other varıables ıncluded ın the analysıs The percent of the explaıned varıance due to the above mentıoned varıables was calculated

The one way analysıs of varıance was used to study the effect of growth on the haemoglobın ın each sex separate ly To study the sex dıfferences ın haemoglobın whıle con trollıng for the growth varıable the *Z* test was used Both technıques were used ın Tables 2 and 3

RESULTS

(*a*) *Haematologıcal results*

The dıstrıbutıon of haemoglobın PCV and MCHC for the whole communıty of chıldren ıs presented ın Fıg 1

Haemoglobın The mean haemoglobın ıs 11 1 g/100 ml S D 1 2 (*n*=858) A haemo globın level below 9 g/100 ml was found ın 3 8% of chıldren below 10 g/100 ml ın 13 5% and below 11 g/100 ml ın 37 3%

Packed cell volume The mean PCV ıs 36 1 S D 2 8 (*n*=845) A haematocrıt level below 34 was found ın 16 8% of chıldren

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(*b*) *Effect of socıocultural background on the haemoglobın level*

The separate effect of socıal class of father educatıon and regıon of orıgın of mother bırth

Table 1 *The effect of socıal class of father educatıon and regıon of orıgın of mother bırth rank and sex of chıld on the haemoglobın at 9 month of age*

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Bırth rank				
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Submitted Sept 30 1976
Accepted Jan 18 1977

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cates that the prevalence of a level below 11 g/100 ml ıs 37%, but only 13 5% of these fall below 10 g/100 ml Therefore most of the ınfants consıdered anemıc by the WHO definıtıon of anemıa 11 g/100 ml (11) have a mıld anemıa The PCV determınatıon and the calculatıon of the MCHC ındıcate that the anemıa ıs most probably due to ıron deficıency Iron deficıency was shown as the maın cause of anemıa ın ınfancy and early chıldhood ın Israelı chıldren from an ımmıgrant town settlement (6) Low ıron ıntakes durıng the first year of lıfe have been demonstrated ın a sample of chıldren ın our communıty (7)

The hıgher prevalence of anemıa ın thıs study ın comparıson to those from Brıstol or North Sweden may be due to dıfferences ın the socıoeconomıc factors of the populatıons The communıty presented here ıs maınly socıal class III and below A statıstıcally sıgnıficant dıfference ın the haemoglobın level was shown by socıal class The mean haemoglobın was lower ın the lower socıal class groups

No effect of the bırth rank on the haemoglobın level was noted In the populatıon studıed anemıa of pregnancy ıs ıntensıvely treated (8) Maıntenance of normal levels of haemoglobın durıng pregnancy may have amelıorated the effect of successıve bırth on the development of anemıa ın ınfancy

No assocıatıon between bırth weıght and length and mean weıght ıncrement per week and haemoglobın level at nıne month of age could be shown for each sex separately Burman (2) demonstrated the effect of bırth weıght on haemoglobın up tıll the age of three month but none ın the later age groups It was postulated by Beal (1) that ınfants wıth hıgh ıncrements have ıncreased appetıtes and theır ıron ıntake per kg ıs the same as that of ınfant wıth lower ıncrements

The sex dıfferences ın haemoglobın level were hıghly sıgnıficant Are these dıfferences due to dıfferences ın growth? Bırth weıght was hıgher ın males than ın females ($p<0\ 003$) The analysıs of the mean haemoglobın by bırth weıght ındıcated that ın each sex the dıfferences are not sıgnıficant, but ın the categorıes of bırth weıght males have persıstently lower mean haemoglobın than females In addıtıon whıle studyıng mean weıght ıncrement per week from bırth to nıne month males have lower mean haemoglobın levels ın the same categorıes of weıght ıncrement than females thıs dıfference ıs of hıgh statıstıcal sıgnıficance The conclusıon from these findıngs are that the dıfferences ın haemoblobın level by sex are not related to growth Investıgatıon of susceptıbılıty to ınfectıon by sex and ıts relatıonshıp to anemıa mıght throw further lıght on the above mentıoned sex dıfferences

The five varıables studıed explaın only 6% of the varıance therefore further factors have to be studıed lıke ıron ıntake sources of ıron ın the dıet blood loss and the common ıllnesses of ınfancy lıke dıarrheal and upper respıratory dıseases

ACKNOWLEDGEMENT

We thank Prof S L Kark Head of the Department of Socıal Medıcıne of the Hebrew Unıversıty Hadassah Medıcal School and Chaırman of the program of development for hıs support and advıce Dr Z Shamır Head of the Mother and Chıld Health unıt and the staff from Kıryat Hayovel Communıty Health Center of the Department of Socıal Medıcıne who took part ın the surveıllance

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CASE REPORT

FAILURE OF DETERMINATION OF FETAL LUNG MATURATION DUE TO FALSE NEGATIVE LECITHIN/SPHINGOMYELIN RATIO IN A CASE OF PIERRE ROBIN SYNDROME

D PELEG J GOLDMAN J BOYANOVER and E ELIAN

From the Department of Obstetrics and Gynecology and Department of Pediatrics Hasharon Hospital Petah Tikva and Tel Aviv University Medical School Tel Aviv Israel

ABSTRACT Peleg D Goldman J Boyanover J and Elian E (Department of Obstetrics-Gynecology and Department of Pediatrics Hasharon Hospital Petah Tikva and Tel Aviv University Medical School Tel Aviv Israel) Failure of determination of fetal lung maturation due to false negative lecithin/sphingomyelin ratio in a case of Pierre Robin syndrome Acta Paediatr Scand 66 519 1977 —A case is reported of a baby with Pierre Robin syndrome born at term with adequate lung maturity The evaluation of fetal lung maturity before birth by means of the lecithin/sphingomyelin ratio and foam test in the amniotic fluid revealed immaturity of the lungs Examination of the tracheal aspirate following delivery however showed adequate lung maturity in the neonate

KEY WORDS L/S ratio foam test lung maturity amniotic fluid tracheal aspirate Pierre Robin syndrome

Several methods have been suggested for the evaluation of fetal lung maturity by means of the amniotic fluid One of the well accepted methods is he determination of lecithin/sphingomyelin (L/S) ratio (5) Since this test requires specialized manpower and equipment simpler tests such as the rapid foam or bubble test have been used This test is also rather reliable (4) Recently both the tracheal aspirate and gastric fluid in the newborn baby have been found to be well suited for the determination of both these tests (1 2 7) As shown by Giudicelli et al (6) most of the phospholipids present in tracheal aspirate and amniotic gluid are identical in proportion and fatty acid composition

Nevertheless in spite of the reliability of the foam test and the L/S ratio in the amniotic fluid in certain conditions they are unreliable The doubtful cases include staining of the fluid by blood or meconium in diabetes mellitus of the mother complicating pregnancy (3) and in other conditions Moreover little has been said in the literature on the subject of congenital malformations which do not permit the determination of lung maturity in the amniotic fluid as for example may be demonstrated in the syndrome of Pierre Robin This type of anomaly may be concerned with the impeded passage of lecithins from the fetal lungs into the amniotic fluid most probably due to hyperextension of the baby s head and prolapse of the tongue

CASE REPORT

A 23 year-old woman with normal pregnancy and delivery in the past was admitted to the delivery floor of Sharon Hospital in the 40th week of her second pregnancy On examination mild preeclamptic toxemia and suspected breech presentation X ray examination confirmed the breech position with marked extension of the head In view of this finding amniocentesis with fetography was performed by means of Pantopaque (ethyl iodophenylundecanoate) and Lipiodol (sodium diatrizoate meglumine diatrizoate) The latter failed to reveal evidence of a cervical tumor as a cause for hyperextension or other external fetal abnormalities Repeat X rays 12 and 24 hours after amniocentesis and injection of the contrast material proved the lack of swallowed opaque fluid by the fetus Foam test performed as a routine test for the determination of fetal lung maturity was negative in all dilutions while the L/S ratio was 1 0

Acta Pædiatr Scand 66 519–520 1977

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The L/S ratio was determined as follows After extraction in a mixture of chloroform and methanol and precipitation in acetone phospholipids were separated on silica gel G plates The separated sphingomyelin and lecithin were collected from the plates their ratio being determined by the phosphate ratio determination The method is based on the reaction between phospho ammoniummolybdate and α aminonaphtosulfonic acid reagent Creatinine and uric aicd levels showed good fetal maturity

Following admission uterine concentrations started however in view of the fetal presentation Caesarean section was performed When the uterine cavity was opened an exceptionally large amount of clear amniotic fluid was found The fetus was seen to be in breech presentation with marked hyperextension of the head and the umbilical cord twice around the baby s neck The baby was delivered in satisfactory condition with Apgar score of 8 after 1 min and 9 after 5 min Immediately following delivery the fluid from the trachea and pharynx were sucked by means of a mucus extractor the foam test was done in this aspirate and found to be positive in all dilutions The L/S ratio was 3 2 Physical examination of the neonate revealed the typical triad including gueule de loup and micrognatia corresponding to the Pierre Robin syndrome The baby remained with a hyperextended head for several days showing no signs of repsiratory distress

COMMENT

A newborn with Pierre Robin syndrome has been described in whom the connection between the fetal lungs and the amniotic fluid in the uterine cavity was obstructed possibly due to prolapse of the tongue The intrauterine obstruction of the linkage between the fetal laryngopharynx and the amniotic fluid may be evidenced by hydramnion and the faulty swallowing of contrast material during fetography Furthermore, it is probable that this obstruction resulted in failure of the foam test and L/S ratio in the amniotic fluid to demonstrate fetal lung maturity In fact this block between the respiratory system of the fetus and the amniotic cavity may have resulted in the reduction of lecithins in the amniotic fluid Nevertheless when the baby was delivered the true lung maturity of the infant could be determined in the trecheal aspirate by means of the mentioned tests indeed good lung maturity was found in the baby

This case stresses the significance of the determination of fetal lung maturity as evaluated by means of the foam test and L/S ratio in the tracheal aspirate This determination offers the present and true lung maturity independently of the free flow between fetal lungs and its environment

It is possible that in other malformations of the laryngopharynx a similar failure of evaluation of lung maturity in the amniotic fluid may be found Consequently attention is drawn to the fact that in case of negative L/S ratio in the amniotic fluid in term pregnancy other than a false negative test a fetal anomaly should be suspected, blocking the exchange between the fetal lungs and the amniotic cavity

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Submitted June 4 1976
Accepted Nov 5 1976

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Acta Pædiatr Scand 66 521–524 1977

CASE REPORT

ECHOCARDIOGRAPHIC DIAGNOSIS OF AN ANEURYSM OF THE MEMBRANOUS VENTRICULAR SEPTUM

J H FAST and R J MOENE

From the Department of Cardiology Free University Hospital Amsterdam The Netherlands

ABSTRACT Fast J H and Moene R J (Department of Cardiology Free University Hospital Amsterdam The Netherlands) Echocardiographic diagnosis of an aneurysm of the membranous ventricular septum Acta Paediatr Scand 66 521 1977 —A patient with a small ventricular septal defect complicated by an aneurysm of the membranous ventricular septum is reported The aneurysm was diagnosed by left ventricular angiocardiography and non invasively by echocardiography The clinical significance of the anomaly is discussed in relation to the pertinent literature

KEY WORDS Aneurysm of the membranous ventricular septum echocardiography

Aneurysm of the membranous portion of the ventricular septum although an unusual lesion should be recognized in an early stage since it may accompany spontaneous diminution in the size of an associated membranous ventricular septal defect and in some cases may be a prelude to spontaneous closure of ventricular septal defects (9 12) Until recently selective angiocardiography was the only reliable (non surgical) means of diagnosing the anomaly during life as there are no specific diagnostic findings on physical examination electrocardiography or radiography

This report emphasizes the value of echocardiography as a non invasive means of diagnosing this lesion

CASE REPORT

The patient a boy was born on April 10 1973 after a full term uneventful pregnancy At the age of 3 months he was admitted to the hospital because of tachypnoea and the discovery of a heart murmur

Physical examination revealed a dyspnoeic infant with signs of congestive heart failure the heart was enlarged and the liver was palpable 3 cm below the costal margin Auscultation disclosed a grade 3/6 pansystolic murmur maximal at the left of the sternal border a second heart sound of normal intensity and a third heart sound at the apex The electrocardiogram showed left ventricular hypertrophy the chest X ray revealed a slight prominence of the pulmonary vascular markings and a slightly enlarged heart

The diagnosis of ventricular septal defect was made and heart failure was successfully treated by digitalis

The boy grew without problems and at the age of one year digitalis was discontinued

At the age of 3 years the child was asymptomatic at that time physical examination revealed a grade 3/6 pansystolic murmur without signs of heart failure The electrocardiogram and the chest X ray were normalized Cardiac catheterization demonstrated a small left to-right shunt at the ventricular level with normal cardiac pressures the pulmonary to systemic flow ratio was 1 5 1 A ciné angiocardiogram of the left ventricle showed an aneurysm of the membranous septum with a minimal left to-right shunt (Fig 1)

Using an ECHO cardiovisor with multiscan facilities and placing the transducer in a sagittal LV long axis cross section position the aneurysm was visualized as a mass of echoes protruding in the outflow tract of the right ventricle during the systolic phase of the heart cycle During diastole the aneurysmal bulge was completely absent From the moving picture a single frame taken during systole is reproduced in Fig 2

No operative treatment was recommended but close follow up observation will be carried out

The L/S ratio was determined as follows After extraction in a mixture of chloroform and methanol and precipitation in acetone phospholipids were separated on silica gel G plates The separated sphingomyelin and lecithin were collected from the plates their ratio being determined by the phosphate ratio determination The method is based on the reaction between phospho ammoniummolybdate and α aminonaphtosulfonic acid reagent Creatinine and uric aicd levels showed good fetal maturity

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No operative treatment was recommended but close follow up observation will be carried out



Fig 1 Left ventricular ciné angiocardiogram in the left anterior oblique position showing an aneurysmal formation of the membranous septum *A A* =ascending aorta *A M S* =aneurysm of the membranous septum *L V* =left ventricle

DISCUSSION

Lev & Saphir (7) attributed the first description of an aneurysm of the ventricular septum to Laennec in 1826 Since the first ante mortem diagnosis by Steinberg in 1957 (11) several cases have been diagnosed by surgery or angiocardiography The incidence of aneurysm of the ventricular septum found at autopsy has been reported to be low (8 11) Varghese et al (12) however reviewed forty eight left ventricular cine angiocardiograms of patients with ventricular septal defects investigated over a 2 year period and found 16 aneurysms of the membranous septum The majority of aneurysms occurred in the presence of a small ventricular septal defect A high incidence was recently also reported by Freedom et al (4)

Mall postulated a congenital origin of these aneurysms and several other authors accepted the concept of a congenital origin (4)

This hypothesis is not supported by recent reports (6 12) in which aneurysm formation is ascribed to or described to occur in association with spontaneous closure of ventricular septal defects Spontaneous closure of muscular defects may occur by apposition of the margins of the defect (13) Many membranous defects also close spontaneously the mechanisms are not yet definitely understood however The only documented mode of closure of these defects is adherence of the medial leaflet of the tricuspid valve to the margins of the defect (2) In addition there is some evidence that endocardial proliferation stimulated by turbulent blood flow through the defect, may cause closure of a membranous defect (13) The weakness of this newly formed tissue may cause it to bulge into the right ventricular cavity as a result of the higher left ventricular pressure (10) Varghese et al (12) reported a proved case of aneurysm of the membranous septum with a ventricular septal defect which later closed spontaneously

This observation together with the frequent association of aneurysms with small ventricular defects strongly suggests that aneurysmal formation may be a prelude to spontaneous closure of these defects

The patient presented here was in congestive heart failure at the age of 3 months at the age of 3 years he was asymptomatic and the electrocardiogram and the chest X ray were normalized suggesting a diminution of the size of the defect In this regard the demonstration of a septal aneurysm in patients with a ventricular septal defect is an additional sign of reduced defect size According to some authors (4 5) certain phonocardiographic features are most suggestive of an aneurysm with associated septal defect i e an early systolic click and late systolic accentuation of the VSD murmur In our patient these signs were absent and the aneurysm was demonstrated by left ventricular angiocardiography

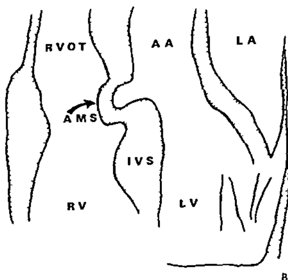



Fig 2 (A) Echocardiographic picture of the aneurysms of the membranous septum (B) Schematic drawing of the echocardiographic picture *A A* =ascending aorta *A M S* =aneurysm of the membranous septum *I V S* = interventricular septum *L A* =left atrium *L V* =left ventricle *R V* =right ventricle *R V O T* =right ventricular outflow tract

Real time two dimensional ultrasonic cardiac visaging permits a non invasive accurate study of the complete movement of the interventricular septum In this patient a mass of echoes was noted near the base of the septum at the place of the aneurysm

The documentation given in the present report (Fig 2) is still more convincing when viewing the moving picture Assad Morell et al (1) used the single element echocardiographic technique to demonstrate the presence of an aneurysm of the membranous septum This technique gives pictures of a higher quality than the two-dimensional technique because of the poor resolution in the presently available real time two-dimensional picture However when viewing the two-dimensional picture in real time the important information of motion in the picture is added

Since rupture endocarditis thromboembolism obstruction of the right ventricular outflow aortic insufficiency and conduction disturbances have been documented as complications of septal aneurysms (10) a long term follow up of these patients is needed to evaluate the natural course of this anomaly In this respect echocardiography is an elegant tool for the assessing of the presence and the dimension of septal aneurysms

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Submitted Oct 11 1976
Accepted Dec 22 1976

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Acta Pædiatr Scand 66 525–528 1977

CASE REPORT

T3 TOXICOSIS IN CHILDREN

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ABSTRACT Harland P C McArthur R G and Fawcett D M (Division of Paediatrics Faculty of Medicine University of Calgary Calgary Alberta) T3 toxicosis in children Acta Paediatr Scand 66 525 1977.—Triiodothyronine (T3) toxicity has been well documented in adults but only isolated cases have been reported in children Two girls presented with firm goitres and overt hyperthyroidism In each patient total serum thyroxine (T4) values by competitive protein binding were normal however total T3 values by radioimmunoassay were elevated One patient had Graves disease the second patient had Hashimoto s disease which has been only infrequently associated with T3 toxicity in adults Both patients responded to therapy with propylthiouracil The mechanisms by which T3 is preferentially secreted in thyrotoxic states in man are poorly understood but iodine deficiency and poor iodination of thyroglobulin may be important factors

KEY WORDS Children T3 thyrotoxicosis Hashimoto s disease

The recent development of a simple and rapid immunoassay for triiodothyronine (T3) in serum (7 8 9) has opened interest in the contribution of this hormone in thyrotoxic states T3 toxicity in the presence of normal serum thyroxine (T4) has been well documented in adults (13 15 25) In the paediatric literature however only isolated cases have been reported (17 22) suggesting that this disorder may be less frequent in childhood

Two children are reported who demonstrated an elevation in T3 Each was referred as a diagnostic problem in that their clinical findings suggested thyrotoxicosis but the usual thyroid function tests were normal

METHODS

Total serum T4 was measured by the Ames tetralute method (University of Alberta Edmonton) and by competitive protein binding analysis (Bio-Science Laboratories Van Nuys California)

Serum free T4 was measured with the aid of magnesium precipitation and radioimmunoassay (Bio Science Laboratories Van Nuys California)

Total serum T3 was measured by radioimmunoassay (Bio-Science Laboratories Van Nuys California)

Antithyroglobulin antibody titres and thyroid binding globulin (T B G) were measured by Bio Science Laboratories Van Nuys California [131]I uptakes were determined by standard methods

REPORT OF CASES

Patient 1 This 10 year-old girl was found to be thyrotoxic during hospital admission for infectious hepatitis For 6 months prior to admission she had noticed heat intolerance emotional lability sweaty palms and palpitations Examination revealed a pulse of 110 *per minute fine tremor of the hands* overt exophthalmos and an enlarged firm irregular thyroid twice normal size weighing approximately 50 g The blood pressure was 115/60 There was no family history of thyroid disease

Investigations (Table 1) showed a normal T4 (6 4 µg/100 ml) elevated T3 (266 ng/100 ml) and a high [131]I uptake (71% at 4 hours) Antithyroglobulin antibodies were present in a titre of 1 2 500 and the T B G was normal (15 µg/100 ml) Typical *Hashimoto* s disease was later reported on thyroid biopsy

Table 1 *Thyroid function in 2 children with T3 toxicosis*

Test	T4 by C P B µg/100 ml	Free T4 (ng/100 ml)	T3 by R I A (ng/100 ml)	¹³¹I uptake (%)	Thyroid antibody titre	T B G (µg T4/100 ml)	Biopsy
Normal	3 0–7 0	2 7–5 7	60–190	4 hrs 5–15 24 hrs 10–35	<1 16	10–26	
Pt I	6 4	7 4	266	4 hrs 21 24 hrs 36	1 2 500	15	Hashimoto s
Pt II	6 2	6 3	372	4 hrs 17 24 hrs 33	1 32	20	Graves

She was treated with propylthiouracil 50 mg q 8 h for 2 years Two months following the initiation of therapy symptoms had improved and the serum T3 value was normal (112 ng/100 ml) One year after the discontinuation of propylthiouracil she remains well with a normal serum T3 level

Patient II This girl aged 10 years gave a 1 year history of increasing restlessness tremor of the hands emotional lability and enlargement of the thyroid Her mother had 2 operations for Graves disease at age 9 and 10 years Examination revealed an anxious fidgety girl with mild exophthalmos and a pulse of 110/minute The thyroid gland was firm diffusely enlarged and felt 3 times normal size weighing approximately 75 g The blood pressure was 115/60

Thyroid studies (Table 1) showed a normal T4 (6 2 µg/100 ml) The free T4 was marginally elevated (6 3 ng/100 ml) and the total T3 was elevated at 372 ng/100 ml Antithyroglobulin antibodies were present in a titre of 1 32 and T B G was normal (20 µg/100 ml) Thyroid biopsy showed histology compatible with Graves disease

She was initially treated with propylthiouracil 100 mg every 8 hours for 2 months She was subsequently maintained on 75 mg every 8 hours for 2 years Two months following the initiation of therapy she appeared euthyroid and her serum T3 level was 146 ng/100 ml Six months after the discontinuation of propylthiuracil therapy she remains well with a normal serum T3 level

DISCUSSION

We believe that our patients exhibited true T3 toxicosis although we recognize that the T3 levels in our subjects are not as high as may be found in adults with T3 toxicosis (13 15 25) and that normal values for serum T3 are higher in children than adults (23) In one reported paediatric case the T3 level was 1 200 ng/ml (17) The free T4 values though above the normal range in our 2 patients are not as high as levels usually seen in T4 thyrotoxic children Also elevated were the radioactive iodine uptake levels at 4 and 24 hours This is consistent with adult reports of T3 toxicity Normal binding of T4 was confirmed by normal T B G levels The biopsy results showed that one patient had Hashimoto s disease which has been only infrequently associated with T3 toxicosis in adults The other girl with lower antibody titres had Graves disease confirming reports (2, 16) that antibodies may be found in this disease

The circulating pool of T3 in man is derived from 2 sources (Fig 1) 1) the thyroid gland and 2) the peripheral conversion of T4 to T3 (4 10 11 21 24) T3 in euthyroid subjects is secreted in smaller quantities than thyroxine, however its significance is heightened by its greater calorigenic effect estimated to be 3 to 4 times more potent than T4 (19 18)

The advent of radioimmunoassay in measuring serum T3 and T4 has delineated the entity of T3 toxicosis accurately and allowed investigators to evaluate its importance in thyroid disease There appears to be a wide biochemical spectrum in the ability of the hyperactive thyroid gland to secrete variable quantities of either hormone and those patients in which T3 alone is elevated represent a variation in the spectrum

Both T4 and T3 are normally synthesized at the same rate (10) T3 is formed by the coupling of monoiodotyrosine (MIT) to diiodotyrosine (DIT) and T4 by the coupling of 2 DIT residues It would be logical that the formation of T3 and T4 depend upon the relative availability of MIT and DIT

Data from animal studies indicate that the normal thyroid increases secretion of T3 rela

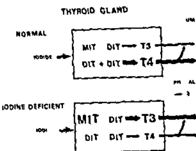


Fig 1 Variations in production of T3 in normal and iodine deficient subjects

tive to T4 during iodine deficiency Rats fed a low iodine diet increase the MIT/DIT ratio in newly formed thyroglobulin within 24 hours (26) such that within 2 weeks the T3/T4 ratio has also increased That similar factors (Fig 1) predispose to preferential secretion of T3 in man is supported by the high incidence of T3 toxicity in iodine deficient areas (14 20)

It is therefore attractive to consider that relatively poor iodination of thyroglobulin occurred in our patients resulting in preferential secretion of the more potent T3 This has been readily demonstrated in patients following thyroid irradiation where there is a relative block in organic binding of iodine and a decrease in overall iodine accumulation (3) Similar abnormalities in intrathyroidal iodine have been demonstrated in Hashimoto s disease (27 5) Elevated T3 levels in Graves disease may be explained on the basis that high serum iodine inhibits intrathyroidal organification of iodine and blocks release of T4 (12)

T3 in the serum may also be derived from non thyroidal sources Evidence suggests that in normal (24) or hypothyroid (4) patients exogenous T4 is converted to T3 Chopra (6) recently estimated that 3/4 of circulating T3 arises from the peripheral conversion of T4 to T3 This again raises the question whether T4 is primarily active or exerts its metabolic effect only after deiodination to T3

The incidence of T3 thyrotoxicosis in adults has been estimated at 5–10% (14 1) of all thyrotoxic patients In iodine deficient areas this is much higher (14 20) Too little information is presently available to determine the true incidence of T3 toxicosis in children

In summary two thyrotoxic children were investigated In each total serum T4 and thyroxine binding globulin were normal while total serum T3 levels were elevated Our cases demonstrate that T3 toxicosis must be suspected when the patient appears thyrotoxic but routine thyroid function tests fail to support the clinical diagnosis

ACKNOWLEDGEMENTS

We are grateful to Mrs Joyce Cunningham for excellent secretarial assistance and to Mrs Trudy Jorgensen for her assistance in the preparation of the illustrations

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Data from animal studies indicate that the normal thyroid increases secretion of T3 rela

Acta Pædiatr Scand 66 529–534 1977

CASE RFPORT

CARBAMYL PHOSPHATE SYNTHETASE DEFICIENCY WITH NEONATAL ONSET OF SYMPTOMS

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ABSTRACT Farriaux J P Ponte Cl Pollitt R J Lequien P Formstecher P and Dhondt J L (Service de Genetique et Maladies Hereditaires du Metabolisme de l Enfant Cite Hospitaliere Lille Hopital Calmette Lille France and M R C Unit for Metabolic Studies in Psychiatry Sheffield UK) Carbamyl phosphate synthetase deficiency with neonatal onset of symptoms Acta Paediatr Scand 66 529 1977 —The clinical course and biochemical findings in a case of carbamyl phosphate-synthetase deficiency are described The patient a boy presented 48 h after birth with rapidly developing hypotonia and hypothermia Pulmonary haemorrhage melaena and haematemesis ensued and despite ventilatory assistance and peritoneal dialysis the patient died on the fifth day A virtual absence of carbamyl phosphate synthetase I (N acetylglutamate dependent) was proved by analysis of tissue samples removed post mortem Other urea cycle enzymes were normal

KEY WORDS Urea cycle hyperammonaemia

Carbamyl phosphate synthetase (CPS) deficiency is one of the more recently described (12) of the hereditary anomalies of the urea cycle enzymes It is also one of the least common (8 cases) particularly as among the observations presented as CPS deficiency some appear debatable including the original case described by Freeman et al (12) Significantly decreased levels of hepatic CPS can be encountered in other conditions—Reye s syndrome (29) non ketotic hyperglycinaemia (22) hyperornithinaemia with hyperammonaemia and homocitrullinuria (13) methylmalonic acidaemia (18 20) and propionic acidaemia (6) We have borne this point in mind in the investigation of the present case the second to be reported in detail

CASE REPORT

The patient was the second child of young (25 and 26 y) healthy non-consanguinous parents with no family history of illness Their first child a girl was born in 197- and had been healthy except for an isolated and unexplained convulsive episode in 1975 The patient a boy (R J) was born at term after an uneventful pregnancy and confinement He weighed 3 700 g was 52 cm long and had Apgar scores of 10 at 1 and at 5 min For the first 48 h of life he presented no problems but then became fretful hypotonic and hypothermic He was transferred to the neonatal intensive care unit where on admission at 56 h of age he was found to be drowsy and hypotonic with loss of primitive reflexes Despite intravenous administration of 10% glucose solution and antibiotics and feeding by gavage with donated human milk and Lacmil (8×20 ml) the patient s condition progressively worsened and irregular laboured breathing developed Ventilatory assistance was started at 66 h The child remained hypotonic with hyperactive tendon reflexes and his general condition deteriorated until by 78 h he was cyanosed and grey The pharyngeal aspirate contained fresh blood and there was haematemesis and melaena All alimentation was then withdrawn and peritoneal dialysis begun with 17 cycles of 100 ml between the 78th and 93rd hours This had no clinical effect and the child s condition remained unchanged until death in the 104th hour of life

LABORATORY METHODS

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Table 3 *Carbamyl phosphate synthetase activity (μmol/h/g tissue) in post mortem tissue specimens (R J) (^{14}C method)*

The results in brackets were obtained with the addition of OCT in the form of a homogenate of the patient s liver correcting for residual CPS activity

	With *N* acetyl glutamate	Without *N* acetyl glutamate
Liver	0 140	0 090
Intestine	0 0 0 (0 0 6)	0 070
Brain	<0 001 (0 002)	<0 001
Kidney	0 070 (0 078)	0 070
Control (rat liver)	49 7	1 45

9–44 μmol/l) 1488 μmol/l at 78 h and 1048 μmol/l after peritoneal dialysis The plasma amino acid pattern (Table 1) was abnormal with an almost complete absence of citrulline and considerable increases in a number of amino acids The cerebrospinal fluid also showed a highly abnormal amino acid composition (Table 1) There was a normal urinary excretion of orotic acid (0 16 mg/ml equal to 0 20 mg/mg creatinine) and no abnormal increase in argininosuccinic propionic methylmalonic or isovaleric acid excretion

Enzyme analysis on tissues removed within minutes of death showed a virtual absence of CPS with normal levels of the other urea cycle enzymes (Table 2) The radiochemical determination of CPS (Table 3) permitted the conclusion that the residual activity in liver about 0 25% of normal was due largely to CPS II (Cytoplasmic CPS—glutamine dependent) as activity was only slightly reduced on omission of *N* acetylglutamate from the incubation mixture Cultured skin fibroblasts from the patient gave CPS activities (with addition of liver homogenate) of 0 005 and 0 007 μmol/h/g of packed cells with and without *N* acetylglutamate respectively A single control sample gave 0 001 μmol/h/g in each case The CPS I activity of these fibroblasts was thus extremely low or zero

The blood urea level was low at 2 mmol/l (0 12 g/l) The serum showed raised levels of enzymes—glutamate oxaloacetate transaminase 145 I U (normal <40), lactate dehydrogenase 600 I U (normal <200) and alkaline phosphatase 117 I U (normal <70) Other investigations showed a lowering of coagulation factors (VII+X V and VIII) to between 34 and 50% of normal and in the E E G signs of severe irritation with epileptic discharges on both left and right

The patient s parents showed normal blood ammonia and amino acid levels and normal urinary amino acids and orotic acid both before and after the ammonium chloride load

DISCUSSION

Clinically two main types of inherited CPS deficiency seem to be distinguishable (Table 4) Firstly there is the neonatal form with a virtual absence of CPS I and characterized by a rapidly fatal outcome with very marked hyperammonaemia (above 500 μmol/l) Four cases of this variant are now known (14 23[1] 27 and the present case) The second variant the slow form carries only a partial deficiency of CPS I with residual activity greater than 10% The survival in these cases is more prolonged with repeated acute episodes and above all a progressive encephalopathy Hyperammonaemia is always more moderate (below 250 μmol/l)

While the first group is clearly defined the second group is more heterogeneous and was divided by Hsia (17) on the basis of cases reported up to 1974 into two sub groups The clinical and biochemical findings are not in themselves particularly specific and a diagnosis of partial CPS deficiency as the primary defect cannot be made until other entities of similar semiology have been excluded Thus in the first reported case of CPS deficiency that of Freeman et al (12) the presence of ketotic acidosis and neutropaenia is

[1] We thank Dr Palmer for sending us unpublished details of this case

Table 1 *Amino acid levels in blood and cerebrospinal fluid*
Results are in mmol/litre (±standard deviation)

	Blood			Cerebrospinal fluid	
	Patient				
	Day 3	Day 5	Controls (n=14) (age 0–8 days)	Patient Day 5	Controls (n=6) (age 0–1 month)
Aspartate	–	0 78	0 04 ±0 02	0 018	0 001 ±0 001
Threonine	0 10	0 07	0 12 ±0 06	0 082	0 035 ±0 022
Serine	0 15	0 19	0 21 ±0 11	0 20	0 045 ±0 022
Asparagine / Glutamine	–	3 7	0 87 ±0 34	7 1	0 60 ±0 21
Proline	0 87	0 46	0 26 ±0 13	0 057	0 004 ±0 003
Glutamate	0 67	1 4	0 19 ±0 11	0 14	0 004 ±0 003
Citrulline	–[b]	0 0002	0 011±0 06	0 003	0 0006±0 0005
Glycine	0 21	0 68	0 41 ±0 15	0 11	0 010 ±0 003
Alanine	2 2	2 6	0 38 ±0 19	1 6	0 036 ±0 015
γ Aminobutyrate	–[b]	0 17	0 011±0 012	0 14	0 001 ±0 000
Valine	0 16	0 23	0 16 ±0 06	0 21	0 011 ±0 004
Methionine	0 030	0 20	0 044±0 039	0 096	0 002 ±0 002
Isoleucine	0 060	0 065	0 045±0 018	0 007	0 004 ±0 002
Leucine	0 19	0 22	0 092±0 036	0 21	0 009 ±0 005
Tyrosine	0 15	0 23	0 084±0 034	0 078	0 011 ±0 002
Phenylalanine	0 020	0 042	0 073±0 024	0 037	0 006 ±0 003
Lysine	–	0 67	0 28 ±0 11	–	
Ornithine	–	0 19	0 18 ±0 04	–	

Determination technically unsatisfactory
[b] Level too low to measure
[c] Insufficient sample for analysis

acids were determined after sulphosalicylic acid deproteinization using ion-exchange chromatography (Beckman Unichrom amino acid analyser) urine and cerebrospinal fluid amino acids were determined similarly without pretreatment of the specimen Urinary orotic acid analysis was by the method of Adachi et al (1) as modified by Rogers & Porter (26) A search for the presence of argininosuccinic acid in the urine was carried out by thin layer chromatography with ninhydrin-cadmium visualization (7) and for propionic methylmalonic and isovaleric acids in urine by gas chromatography (9)

Urea cycle enzymes were determined (*a*) in Lille according to the method of Brown & Cohen (4) as modified by Snodgrass (28) for ornithine carbamyl transferase (OCT) and the colorimetry of citrulline and by Levin et al (21) for the other enzymes (*b*) in the laboratory of Prof Polonovski (by Dr Cathelineau) using the technique previously described (5) and (*c*) in Sheffield by the radioisotopic method of Kerson & Appel (19) after homogenization of the tissues in 0 1% cetyltrimethylammonium bromide

Ammonium chloride tolerance tests on the parents were performed using a single oral dose of 3 g

RESULTS

There was marked hyperammonaemia with levels of 1 537 μmol/l at 71 h of life (normal

Table 2 *Activity of urea cycle enzymes in liver (μmol/h/mg protein)*

	CPS		OCT		ASA synthetase	ASA lyase[b]		Arginase	
Method	1	2	1	2	2	1	2	1	2
Patient R J	<0 08	<0 05	15 3	31	0 25	0 7	1 3	608	82
Controls									
Mean	1 03(n=4)	0 70±0 20	17 6(n=8)	32±5 8	0 25−0 4	1 5 1 7 (n=2)	1 0±0 3	280(n=12)	77±20
Range	0 79−1 31		15 2−21 5					118−1 520	

Column 1 – determined at Lille (see laboratory methods section) Column 2 – determined by L Cathelineau (ref 5) For controls n=11
[b] Erythrocyte argininosuccinate lyase – Patient R J 0 130 μmol/min/g haemoglobin Controls (n=20) 0 088±0 036 μmol/min/g haemoglobin

Progress
Died at 75 h
Died on 4th d
Died at 96 h
Died at 104 h
Clinical improvement with low protein diet Died on 5th m
Died at 7 5 m after a short period of clinical improvement with low protein diet
Ali e Clinical improvement with low protein diet
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Alive (IQ=13)

both these cases terminated in pulmonary haemorrhage The clinical picture is suffi ciently characteristic that the idea of searching for undiagnosed cases in the family history might be considered On the biochemical side the finding of hyperammonaemia with lowered citrulline but without other specific amino acid abnormalities ketosis or abnormal excretion of organic acids suggested blockage in either of the first two steps of the urea cycle (CPS or OCT) as the primary defect The absence of abnormal excretion of orotic acid indicated CPS deficiency (2 23) This probable diag nosis was confirmed by demonstration of an isolated deficiency of CPS I in liver tissues

The amino acid results merit some discus sion The abnormalities in the plasma amino acids in our patient were similar to those de scribed previously (14) and easily explained in terms of disruption of the urea cycle (raised aspartic acid lowered citrulline) the necessity to detoxify ammonia (raised glutamine) and by hepatic insufficiency (raised tyrosine methionine and alanine) The abnormalities of cerebrospinal fluid amino acids are more dif ficult to interpret It is worth noting however that the glycine levels (107 μmol/l) are in the range quoted by Perry et al (25) for glycine encephalopathy and the CSF/plasma ratio of 0 16 in our patient (normal 0 02) is also in the range quoted for non ketotic hyperglycinae mia (25) In such cases with normal or only moderately raised plasma glycine levels the absence of glycine cleavage enzyme may be evident in the brain but not the liver (22 25)

The treatment of the neonatal form of CPS deficiency is of great urgency but the classical methods of peritoneal dialysis or exchange transfusion are of limited effect The modest decrease in blood ammonia levels produced by peritoneal dialysis in our patient confirms the calculation made by Glasgow & Chase (15) on the efficiency of this method However this method has recently been successful applied to a case of argininosuccinic aciduria (11) and it is quite possible that such emergency meas ures followed up by low protein diet or one based on keto-derivatives of amino acids (3) may be successful in the milder CPS deficiency variants

With regard to the mode of inheritance it is noteworthy that the cases of the lethal ne onatal form were male and female (Table 4) This could support a recessive transmission of CPS but unfortunately no indication of hetero-zygote state could be revealed in the parents (this case and ref 14) Although CPS can be detected in leucocytes (30) our preliminary results seem too untrustworthy for hetero-zygote detection Similarly antenatal diag nosis using fibroblasts may be theoretically possible (Nadler personal communication

Table 4 *Clinical and biochemical data of reported cases of carbamyl phosphate synthetase deficiency*

Authors	Sex	Age at on set	Vomiting	Respiratory distress	Coma	Seizure	Neuro-logical abnorm alities	Blood ammonia µM/l	Urea-cycle enzyme activities[b] CPS	Others
Neonatal forms										
Gelehrter & Snodgrass (14)	m	30 h	−	+	+	+	Hypertonia Opisthonos Clonic movements	869 (88)	<10%	Normal
Sheffield et al (27)	m	3 d	−	−	+	−	−	1 174	0 2%	OTC 46%
Oberholzer & Palmer (23)	f	22 h	+	−	+	−	Hypotonia	360 (57)	0%	OTC 50% AS normal AL normal
Present patient	m	48 h	−	+	+	−	Hypotonia Hyper reflexia	1 456 (70)	0 1%	Normal
Delayed onset forms										
Freeman et al (12)	f	10 d	+	−	+	−	−	235 (59)	22%	Normal (arginase 77%)
Hommes et al (16) Ebels (10)	f	20 d	+	−	+	+	Hypore flexia Irregular eye move ments	59 (35)	40%	Normal
Arashima & Matsuda (2)	f	7 d	+	−	−	−	Hypotonia Irregular eye move ments	76 (35)	13%	Normal
Odievre et al (24)	m	6 w	+	−	+	+	Hypotonia Rossolimo sign	206 (29)	<20%	Normal
Batshaw et al (3)	f	3 w	+	−	+	+	Spastic hemipar esis	200	<15%	Normal

Upper limit of normal given in parentheses
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suggestive of propionic acidaemia a diagnosis not definitely eliminated In conditions such as methylmalonic acidaemia and propionic acidaemia the reduced levels of CPS are unexplained but seem likely to be secondary to the other more clear cut enzyme deficiencies Partial CPS deficiency is also reported (together with partial OCT deficiency) in Reye s syndrome (29) which is however quite distinctive clinically Observations such as that of Corbeel et al (8) where the CPS deficiency was associated with an anomaly in the glycine cleavage enzyme are more difficult to classify particularly as among other cases of CPS deficiency there is also some degree of hyperglycinaemia without ketosis

In our case the diagnosis of CPS deficiency appears to follow logically from the clinical and biochemical observations The clinical pattern is that of a child born in good condition and with a trouble free initial period followed by vomiting anorexia neurological signs (somnolence convulsions coma) and later by respiratory distress The outcome of this typical form (previously described in detail only by Gelehrter & Snodgrass (14)) is rapidly fatal Intensive care was ineffectual both in our case and the one of Sheffield et al (27) and

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Submitted Aug 16 1976
Accepted Oct 28 1976

(J P F) Service de Génetique et Maladies Hereditaires du Métabolisme de l Enfant
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Acta Pædiatr Scand 66 535–536 1977

ANNOUNCEMENT

There is overwhelming proof that drugs containing 8 hydroxyquinoline derivatives used in treatment of diarrhea may cause serious neurologic damage. The therapeutic benefit from these drugs has not been shown to be in reasonable proportion to the side effects. According to generally accepted medical principles these drugs should for this reason not be used. We appeal to all pharmaceutical companies which are still selling these drugs to discontinue the sale immediately.

Vasterås and Göteborg Sweden March 1977

On behalf of the Swedish Pediatric Association and its Section for Child Neurology

Torsten Berg and *Bengt Hagberg*

BOOK REVIEWS

C R Osborn & N Roydhouse *The tonsillitis habit* T B & W P Roydhouse Publishers Auckland 1976 100 pp illus $12 00

Ever since a Danish surgeon introduced adenoidectomy a hundred years ago this procedure has been met with enthusiasm and its indications have been broad. Unfortunately they still are. This is also true of tonsillectomy. In the foreword to this book Dr Harrison draws attention to the fact that it is only in the last decade that the rationale and the value of the world's most common surgical procedures have been questioned.

The book is an important contribution to the worldwide debate now in progress concerning the indications of these two operations. Certainly part of the material has been previously published but in this monograph extensive experience has been condensed. The book contains clinical findings and practical advice in the conservative management of reversible lesions of the tonsils. One of the authors contributes histopathological views on the reversible and irreversible forms of the tonsillitis habit. One chapter deals with the special anaesthetic problems in this type of oral surgery another with periodontal diseases and their connection with infection in the tonsils. The possible role of cow's milk intolerance and upper respiratory diseases is also discussed. Both the illustrations and the photographs are of high quality. Every chapter has an extensive list of references and there is a name and subject index.

Although the lines upon which to work in regard to T's and A's" remain somewhat unclear even after reading this book and although some of the problems do not apply to all parts of the world—for instance in the Scandinavian countries tonsillectomy in young children and adenotonsillectomy is unusual—the material is certainly of value to every E N T surgeon. Nor is the tonsillitis habit of only minor interest to the paediatrician.

Christer Carenfelt

J A Kuzemko (ed) *Asthma in children* Pitman Medical Publishing Co Ltd London 1976 127 pp illus £3 50 ISBN 0272794007

The prognosis of asthma in children depends on early diagnosis analysis of etiologic factors treatment and careful management. This short text is devoted to children with asthma and covers all aspects of asthma. There is one chapter about the natural history and epidemiology of the disease. One chapter deals with present opinion on the immunology in asthma. There are chapters about history taking and the various allergy tests both in skin and in blood. Tests of pulmonary function are discussed in a special chapter.

Management and treatment are carefully discussed and much good advice and guidance based on deep understanding and practical experience of the disease are given. The chapter on drugs gives a review of the various drugs and their usefulness starting with adrenalin in 1903 and followed by ephedrine in 1925 theophyllamine in 1938 and corticosteroids in 1948. In 1967 we got the selective β receptor stimulants. At the same time sodium cromoglycate was introduced with its good protective effect in children with asthma. Finally we got the steroid aerosols. The effects and side effects of the drugs are carefully discussed.

The book also comprises an up-to-date bibliography of great value. Although it is a slim volume it gives much valuable information for doctors in paediatric practice.

Einar Stenonius

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G R Osborn & N Roydhouse *The tonsillitis habit* T B & W P Roydhouse Publishers Auckland 1976 100 pp illus $17 00

Ever since a Danish surgeon introduced adenoidectomy a hundred years ago this procedure has been met with enthusiasm and its indications have been broad Unfortunately they still are This is also true of tonsillectomy In the foreword to this book Dr Harrison draws attention to the fact that it is only in the last decade that the rationale and the value of the world s most common surgical procedures have been questioned

The book is an important contribution to the worldwide debate now in progress concerning the indications of these two operations Certainly part of the material has been previously published but in this monograph extensive experience has been condensed The book contains clinical findings and practical advice in the conservative management of reversible lesions of the tonsils One of the authors contributes histopathological views on the reversible and irreversible forms of the tonsillitis habit One chapter deals with the special anaesthetic problems in this type of oral surgery another with periodontal diseases and their connection with infection in the tonsils The possible role of cow s milk intolerance and upper respiratory diseases is also discussed Both the illustrations and the photographs are of high quality Every chapter has an extensive list of references and there is a name and subject index

Although the lines upon which to work in regard to T s and A s remain somewhat unclear even after reading this book and although some of the problems do not apply to all parts of the world—for instance in the Scandinavian countries tonsillectomy in young children and adenotonsillectomy is unusual—the material is certainly of value to every E N T surgeon Nor is the tonsillitis habit of only minor interest to the paediatrician

Christer Carenfelt

J A Kuzemko (ed) *Asthma in children* Pitman Medical Publishing Co Ltd London 1976 1?? pp illus £3 50 ISBN 0 272 79400 7

The prognosis of asthma in children depends on early diagnosis analysis of etiologic factors treatment and careful management This short text is devoted to children with asthma and covers all aspects of asthma There is one chapter about the natural history and epidemiology of the disease One chapter deals with present opinion on the immunology in asthma There are chapters about history taking and the various allergy tests both in skin and in blood Tests of pulmonary function are discussed in a special chapter

Management and treatment are carefully discussed and much good advice and guidance based on deep understanding and practical experience of the disease are given The chapter on drugs gives a review of the various drugs and their usefulness starting with adrenalin in 1903 and followed by ephedrine in 19 5 theophyllamine in 1938 and corticosteroids in 1948 In 1967 we got the selective β_2-receptor stimulants At the same time sodium cromoglycate was introduced with its good protective effect in children with asthma Finally we got the steroid aerosols The effects and side effects of the drugs are carefully discussed

The book also comprises an up to-date bibliography of great value Although it is a slim volume it gives much valuable information for doctors in paediatric practice

Ennor Svenonius

D Bergsma & S H Blondheim (eds) *Bilirubin metabolism in the newborn* (II) Birth Defects Original Article Series *Vol XII No 2 Elsevier* Excerpta Medica Amsterdam 1976 348 pp illus US $40 50

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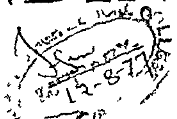
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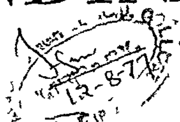
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Acta Pædiatr Scand 66 269–272 1977

ANDERS SPARRMAN AND HIS TRANSLATION OF ROSÉN VON ROSENSTEIN'S TEXTBOOK ON CHILDREN'S DISEASES DURING CAPTAIN COOK'S EXPEDITION TO THE ANTARCTIC REGIONS AND ROUND THE WORLD (1772–1775)

BO VAHLQUIST

Short biography

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Sparrman was elected a member of the Royal Academy of Sciences in 1776 This distinction was soon followed by others making him an honorary member of a number of learned societies both at home and abroad (5) In 1780 he became Curator of the Cabinet of Curiosities (Naturaliekabinettet) of the Academy In 1790 he was appointed Professor of Historia Naturalis and Pharmacy at Stockholm a position which he retained until 1803 At the same time he was also named Assessor of the Collegium Medicum and he still held this position when the Collegium was reorganized in 1813 In his later years he acted as a physician to the indigent in the parish of St Clara in Stockholm He died in 1820



Anders Sparrman in his manhood days holding the position as Curator of the Cabinet of Curiosities at the Royal Academy of Sciences

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Be so kind and remember me with news from Europe especially literary ones Please do promote my translation of Rosen At my return from my journey I will write you a long epistle about it

And after his return to Sweden on August 7 1776

Many and very many thanks for Rosén s translation

In the autumn of 1776 Sparrman went to London for a few months Apparently the translated edition although dated 1776 had still not been published before he left for Swe den Anyhow he does not seem to have procured a copy of the completed work (4) in London because in a letter to Forster of 15 March 1777 he writes

please to send me a copy or two of Roseen I will give one to the King s library

And on March 27 1777

And wish to hear if the Parcel has arrived at Gothenburg and hope I shall see a specimen of my translation of Roseen that I may shew it the Swed Acad of Sciences and present it to the King as another Specimen of my capacity for I am really proud of knowing the Engl language as well as I do and people here look upon it as a terrible difficult thing to write—as it is indeed

On July 25 1777

Roseens book I have been looking at also a little and find that I have forgotten a great deal of English to write it especially Please to excuse my blunders here If you could send me the manuscript of Roseen some time or other I could remember several things better by the cor rections and keep it for my grand Childern if I ever have any *Pray what do the Engl Physicians say of my trans lation of Roseen?*—I was told That Roseen had written a Pharmacopy of 300 pages in 8° only to compositions as for one travelling by land or living in the coun *try—suffi* cient rules it be looked upon here as a Masterpiece the whole medicine comprehended in a nut —Pray is it trans lated into English al eady or would it be wo th wh le at any time

The correspondence continues with a few more letters until Sept 5 1780 but no more reference is made to Rosen s works

Sparrman refers here to Rosen s Hus och rese apoteque (House and Travelapotheque) This work of 11? pages first published in 1765 was enthusiastically received in Sweden and later on translated into Danish and German

Some further notes on Sparrman s life

After his long and adventurous travels in 1772–76 and the following London visit Sparrman went abroad only once more In 1787–88 he was a member of a small group of Swedes travelling on Royal command to West Africa with the aim of finding out whether in the in terior of Senegal or adjacent areas the establishment of a Swedish colony could be meaningful and practicable

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KEY WORDS Henoch-Schonlein s purpura abnormal proteolysis factor XIII

Henoch Schonlein s purpura (anaphylactoid purpura allergic purpura) is characterised by haemorrhagic skin lesions abdominal symptoms including gastro intestinal bleeding renal involvement with proteinuria and haematuria and swelling of joints The symptoms are ascribed to generalised inflammation of the arterioles and capillaries In this syndrome haemostasis assessed with ordinary methods is normal and thereby distinguishes the condition from primary haemorrhagic disorders (17)

It has recently been shown that proteolytic enzymes liberated from inflammatory or damaged tissue cells can specifically degrade certain factors in the coagulation system such as fibrinogen factor V and factor XIII (12 23 24) In patients with erosive gastritis characterised by diffuse haemorrhage from an inflamed gastric mucosa local fibrinolysis in combination with a low factor XIII concentration has recently been shown to contribute to local defect in the patients haemostasis (21)

A similar mechanism was thought to play a role in the pathogenesis of the bleeding symptoms in Henoch Schonlein s purpura We therefore studied a series of patients with this syndrome with special reference to the factor XIII concentration

CLINICAL MATERIAL

The material consisted of 17 consecutive patients (12 boys and 5 girls) aged 2 1/2 to 13 years with Henoch Schonlein s purpura Clinical data and routine haematologic findings are summarised in Table 1 Blood was obtained by puncture of a peripheral vein with the silicone technique and citrated plasma and serum were prepared in the way previously described (19 22)

METHODS AND MATERIALS

Factor VIII clotting activity (F VIII C) was determined according to Nilsson et al (19) factor VIII related antigen

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Table 1 *Haematologic and clinical data*

Pat no	Age (y /mo)	Sex	Hgb (g/l)	Leukocytes ×10⁹/l	Platelets ×10⁹/l	ESR (mm/ 1 h)	Skin lesions	Joint swellings	Abdominal symptoms	Melaena	Renal involvement (proteinuria and/or haematuria)
1	9	M	78	46	262	18	+	+	+	+	+
2	4/6	M	149	7 9	280	32	+	+	+	+	+
3	2/6	M	131– 87	19 0– 9 0	510	26	+	+	+	+	+
4	5/9	M	127	8 1	350	30	+	+	+	+	–
5	5/3	M	119	8 4	310	20	+	+	+	+	–
6	9	F	116	5 2	590	7	+	+	+	+	–
7	6/6	M	112	9 0	280	30	+	+	+	+	+
8	3	M	111– 92	9 0–28 0	440–840	15	+	+	+	+	+
9	6	F	116	6 2	400	47	+	+	+	+	+
10	3	M	103– 66	7 2–18 6	581–690	25	+	+	+	+	+
11	3/6	M	124	6 7	407	33	+	+	–	–	+
12	11	M	139–117	26 0–12 0	171	10	+	+	+	+	+
13	5/9	M	112	8 2–11 8	278–553	56	+	+	+	+	+
14	4/5	F	123	15 0– 5 4	200	36	+	+	–	Not tested	+
15	11/9	M	142	10 6	258	12	+	+	–	Not tested	+
16	3	F	140	5 4	279	28	+	+	–	–	–
17	13	F	148	15 1	330–500	27	+	+	+	–	+
Normal ranges			*115–147*	*6–10*	*150–350*	*1–12*					

(F VIIIR AG) immunochemically with Laurell s rocket technique (13) *fibrinogen* according to Nilsson & Olow (20) *factor XIII* (fibrin stabilising factor = FSF) according to Lorand et al (16) as modified by Henriksson et al (11) and expressed as FSF units/l citrated plasma ×10³ *fibrinolytic activity* in the circulating blood on fibrin plates (resuspended euglobulin precipitate) according to Nilsson & Olow (20) and *fibrin/fibrinogen degradation products (FDP)* with the immunochemical method of Nilehn (18) The FDP were determined in serum obtained from blood collected in tubes containing thrombin (3 NIH U/ml blood) and EACA (5–10 mg/ml blood) *ethanol gelation test* according to Godal et al (8) *plasminogen* with an immunochemical method according to Ganrot & Nilehn (7) *platelet counts* according to Bjorkman (1) *α_2-macroglobulin (α_2M)* as described by Ganrot (6) and *antithrombin III (AT III)* immunochemically (9) *Factor XIII concentrate* factor XIII rich fibrinogen (Fibrinogen Kabi 1 g/100 ml 48 FSF U/ml and fraction I-0 (AHF Kabi 153 FSF U/ml)

RESULTS

The results of co1gulation and fibrinolytic studies are summarised in Table 2 *Factor XIII was low* (below 12 FSF U/l×10³) in 13 of the 17 patients F VIII C and F VIIIR AG were within normal limits or slightly increased The *plasminogen* levels were normal except in the severely affected child (No 1) which is described separately below No increased *fibrinolytic activity* in blood was observed on fibrin plates except in one case (No 7) *FDP* appeared in small amounts in serum from 2 patients including the one with

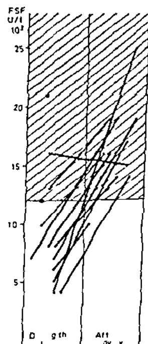


Fig 1 Factor XIII levels during the acute phase of the disease and after recovery

Table 2 *Coagulation and fibrinolytic data*

Case no	Factor XIII FSF units/l ×10³	Factor VIII F VIII C (%)	Factor VIII F VIII R AG (%)	Factor V (%)	Plasminogen (%)	Fibrinogen (g/l)	Fibrinolytic act on fibrin plates (mm)	FDP (mg/l)	α_2M (%)	AT III (%)
1	4	110	95	93	23	1 6	25	0	7?	46
?	8	88	99	85	105	3 1	185	0	?47	100
3	4	110	135	95	70	3 1	10	17	98	78
4	10	90	86	98	1?5	3 7	64	5	231	100
5	8	163	136	96	130	3 7	143	0	?01	–
6	6	80	146	140	1?0	3 8	93	0	155	96
7	7	194	188	101	80	3 9	?75	1?	140	100
8	5	–	–	–	–	–	–	–	–	–
9	13	?05	108	89	105	3 7	171	0	?35	108
10	16	–	–	–	–	–	–	–	–	–
11	1?	–	–	–	–	–	–	–	–	–
1?	3	–	–	–	–	–	–	–	–	–
13	4	–	–	–	–	–	–	–	–	–
14	?	–	–	–	–	–	–	–	–	–
1?	7	–	–	–	–	–	–	–	–	–
16	?1	–	–	–	–	–	–	–	–	–
17	7	?95	?1?	116	135	9 ?	107	0	154	11?
Normal values	1 –18	60–160	60–175	80–1 0	70–130	2 0–4 0	98±54	≤5	150–250 (age matched controls)	60–140

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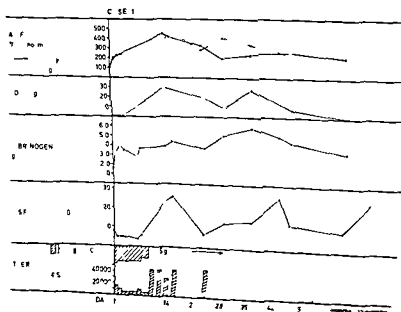


Fig 1 The course of the disease (case 1)

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1	9	M	78	46	262	18	+	+	+	+	+
2	4/6	M	149	7 9	280	32	+	+	+	+	+
3	2/6	M	131– 87	19 0– 9 0	510	26	+	+	+	+	+
4	5/9	M	127	8 1	350	30	+	+	+	+	–
5	5/3	M	119	8 4	310	20	+	+	+	+	–
6	9	F	116	5 2	590	7	+	+	+	+	–
7	6/6	M	112	9 0	280	30	+	+	+	+	+
8	3	M	111– 92	9 0–28 0	440–840	15	+	+	+	+	+
9	6	F	116	6 2	400	47	+	+	+	+	+
10	3	M	103– 66	7 2–18 6	581–690	25	+	+	+	+	+
11	3/6	M	124	6 7	407	33	+	+	–	–	+
12	11	M	139–117	26 0–12 0	171	10	+	+	+	+	+
13	5/9	M	112	8 2–11 8	278–553	56	+	+	+	+	+
14	4/5	F	123	15 0– 5 4	200	36	+	+	–	Not tested	+
15	11/9	M	142	10 6	258	12	+	+	–	Not tested	+
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17	13	F	148	15 1	330–500	27	+	+	+	–	+
Normal ranges			115–147	6–10	150–350	1–12					

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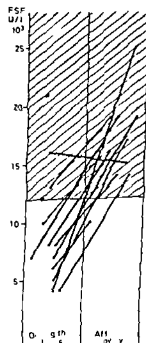


Fig 1 Factor XIII levels during the acute phase of the disease and after recovery

Table 2 *Coagulation and fibrinolytic data*

Case no	Factor XIII FSF units/l ×10	Factor VIII: F VIII C (%)	Factor VIII: F VIII R AG (%)	Factor V (%)	Plasminogen (%)	Fibrinogen (g/l)	Fibrinolytic act on fibrin plates (mm²)	*FDP* (mg/l)	$\alpha_2 M$ (%)	AT III (%)
1	4	110	95	93	23	1.6	25	0	72	46
2	8	88	99	85	105	3.1	185	0	247	100
3	4	110	135	95	70	3.1	10	17	98	78
4	10	90	86	98	125	3.7	64	5	231	100
5	8	163	136	96	130	3.7	143	0	201	–
6	6	80	146	140	120	3.8	93	0	155	96
7	7	194	188	101	80	3.9	275	12	140	100
8	5	–	–	–	–	–	–	–	–	–
9	13	205	108	89	105	3.7	171	0	235	108
10	*16*	–	–	–	–	–	–	–	–	–
11	12	–	–	–	–	–	–	–	–	–
12	3	–	–	–	–	–	–	–	–	–
13	4	–	–	–	–	–	–	–	–	–
14	2	–	–	–	–	–	–	–	–	–
15	7	–	–	–	–	–	–	–	–	–
16	21	–	–	–	–	–	–	–	–	–
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Normal values	1–18	60–160	60–175	80–120	70–130	2.0–4.0	98±54	≤5	150–250 (age matched controls)	60–140

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Fig 2. The course of the disease (case 1).

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Pat no	Age (y/mo)	Sex	Hgb (g/l)	Leukocytes ×10⁹/l	Platelets ×10⁹/l	ESR (mm/1 h)	Skin lesions	Joint swellings	Abdominal symptoms	Melaena	Renal involvement (proteinuria and/or haematuria)
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5	5/3	M	119	8.4	310	20	+	+	+	+	−
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15	11/9	M	142	10.6	258	12	+	+	−	Not tested	+
16	3	F	140	5.4	279	28	+	+	−	−	−
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Normal ranges			115–147	6–10	150–350	1–12					

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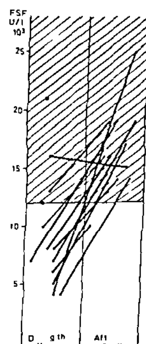


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13	4	–	–	–	–	–	–	–	–	–
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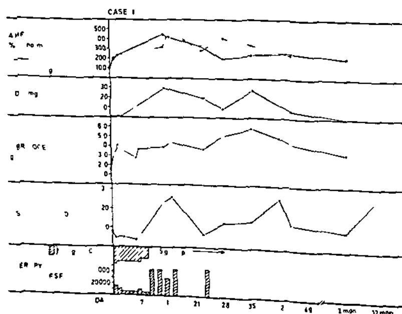


Fig 2 The course of the disease (case 1)

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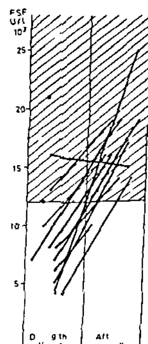


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13	4	–	–	–	–	–	–	–	–	–
14	2	–	–	–	–	–	–	–	–	–
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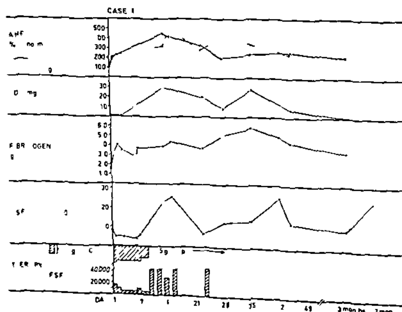


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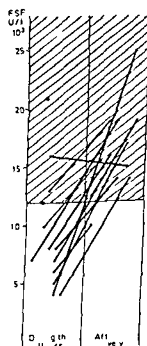


Fig 1 Factor XIII levels during the acute phase of the disease and after recovery

The observations open a new approach to the treatment of severe haemorrhages in these patients The concentrate must be given frequently during the acute phase (Fig 2) owing to the extreme shortening of the half life of factor XIII normally estimated to be 4–5 days (14) and it should be combined with an anti fibrinolytic drug due to the increased susceptibility of fibrin to local fibrinolysis when factor XIII is low

ACKNOWLEDGEMENT

This investigation was supported by grants from the Swedish Medical Research Council (B77 19X-00087 13C) and the Medical Faculty University of Lund

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Submitted Oct 18 1976
Accepted Dec 13 1976

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mal factor XIII concentrations except one patient (No 6) who had 6 FSF U/l×10³ during the acute phase and still showed a subnormal value (10 FSF U/l×10³) after her recovery

Case I

A 9 year-old boy was admitted to our hospital with full blown clinical picture of Henoch Schonlein s purpura and profuse gastro intestinal bleeding He had received 6 units of bank blood within the last 5 hours before admission Various relevant data noted in the course of the disease are given in Fig 2 Therapy was focused on his low factor XIII levels and his raised fibrinolytic activity indicated by the low fibrinogen and plasminogen levels He was thus given factor XIII concentrates (total dose 245 000 U FSF which corresponds to the FSF in about 12 250 ml plasma over the first 3 weeks) Tranexamic acid (AMCA Cyclokapron Kabi) was given parenterally during the first 9 days (total dose 12 g) followed by 6 g a day *by mouth for the following 20 days Immediately* after the beginning of this treatment the gastro intestinal bleeding stopped Factor XIII increased to normal but on withdrawal of factor XIII concentrate for one week it again fell to subnormal level At follow up several months after his recovery the factor XIII level was normal (25 FSF U/l×10³)

DISCUSSION

Factor XIII was low in 13 out of the 17 patients *with Henoch Schonlein s purpura The* normal or slightly decreased values were found in patients who were only mildly ill It seems likely that factor XIII is often low in these patients also in those in whom the haemorrhage is not severe However 5 of the 13 patients who had low factor XIII levels developed anaemia

Though low these factor XIII levels per se are still high enough for normal haemostasis *It has however been shown that in patients* with a low factor XIII level the fibrin clots formed are not fully stabilised and are abnormally susceptible to fibrinolytic attacks (2 5 15) Decreased factor XIII must therefore render the patients more susceptible to local fibrinolysis or other types of abnormal proteolysis and thereby increase the tendency of inflamed mucosa to bleed This mechanism could explain the immediate effect of the treatment *given in case* 1 (factor XIII concentrate and tranexamic acid) Antifibrinolytic treatment therefore seems to be one important measure in the treatment of these patients *The other* and equally important measure is repeated substitution with factor XIII concentrate in order to make sufficient available for fibrin cross linking

Factor XIII has been claimed to decrease in conditions associated with an activation of the coagulation system (3) Of 19 patients with diseases complicated by an abnormal proteolytic activity (fibrinolysis and/or an activated coagulation) we found the factor XIII level to be low in 15 (10) In the present study however no other signs of thrombin activity such as decreased levels of fibrinogen F VIII C and pla*telets and positive ethanol gelation test could* be found In one of our patients (Nr 7) it could not be excluded that a generalised fibrinolytic activity had contributed to the pattern found But other authors have not reported any decrease in factor XIII in fibrinolytic states not even during urokinase treatment (3)

The findings of an exclusive decrease of factor XIII in moderate or severe Henoch Schonlein s purpura suggest some specific degrada*tion of factor XIII Such degradation* has been observed in leukemia (4) erosive gastritis (21) and Weber Christian disease (nonsuppurative nodular panniculitis) (12) Proteolytic enzymes from leukocytes have been found to degrade factor XIII fibrinogen and factor V *in vitro* (24) The low factor XIII levels observed in Henoch Schonlein s purpura might therefore be the result of a destruction by proteases liberated from leukocytes during the inflam*matory* process around innumerable arterioles and capillaries throughout the body The fact that one of the patients (Nr 1) had so low values of factor XIII on admission in spite of the administration of substantial amounts of blood within a short time speaks in favour of an ongoing specific degradation of factor XIII *in the circulation So does* also the persistent high turnover rate of factor XIII in this patient even after the bleeding had stopped thus excluding impaired production as an important cause of the lowness of factor XIII

The observations open a new approach to the treatment of severe haemorrhages in these patients The concentrate must be given fre quently during the acute phase (Fig 2) owing to the extreme shortening of the half life of factor XIII normally estimated to be 4–5 days (14) and it should be combined with an anti fibrinolytic drug due to the increased suscepti bility of fibrin to local fibrinolysis when factor XIII is low

ACKNOWLEDGEMENT

This investigation was supported by grants from the Swedish Medical Research Council (B77 19X-00087 13C) and the Medical Faculty University of Lund

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Submitted Oct 18 1976
Accepted Dec 13 1976

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Acta Pædiatr Scand 66 279–283 1977

CONGENITAL IMMUNODEFICIENCY AND AGRANULOCYTOSIS (RETICULAR DYSGENESIA)

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ABSTRACT Haas R J Niethammer D Goldmann S F Heit W Bienzle U and Kleihauer E (Departments of Paediatrics Universities of Munchen and Ulm Fed Rep Germany) Congenital immunodeficiency and agranulocytosis (Reticular dysgenesia) Acta Paediatr Scand 66 279 1977—A patient is presented who manifested the typical clinical and pathological features of congenital immunodeficiency and agranulocytosis (reticular dysgenesia) Treatment under gnotobiotic conditions enabled the measurement of immunolog ical parameters up to the 17th week of life with the following results negative skin tests low response to phytohaemagglutinin weak response in the mixed leukocyte culture and very few E rosettes Peripheral lymphocytes and lymphocytes in the lymphatic tissues were markedly decreased Humoral immunoglobulins and plasma cells in the organs were decreased The in vitro culture of hemopoietic cells showed a diminished content of myelopoietic progenitor cells (committed stem cells) It is concluded that the disease may be primarily a defect of stem cells with regard to differentiation in myelopoiesis or lymphopoiesis

KEY WORDS Reticular dysgenesia immunodeficiency stem cell defect

In 1959 de Vaal & Seynhaeve (15) described two cases of a disease which they termed reticular dysgenesia A third case was re ported by Gitlin et al (7) and a fourth by Alonso et al (2) These infants died 5 to 15 days after birth Although no immunological studies were carried out necropsy suggested a generalized immune deficiency disorder We have recently observed a newborn with reticu lar dysgenesia The patient lived until the age of 17 weeks under gnotobiotic conditions enabling us to measure immunological para meters

CASE REPORT

Family history

Consanguinity was demonstrated in the family history as shown in Fig 1 Four relatives died during the first year of life as a result of infections but no autopsies were done

Supported by SFB Contract No 112 of Deutsche For schungsgemeinschaft

Patient history

V V was born at term after an entirely uneventful preg nancy with a birth weight of 2500 g Immediately after birth he developed severe diarrhea and was transferred to our hospital at the age of 12 days Examination showed dehydration No thymus was apparent Cultures from the skin body orifices faeces and urine grew numerous or ganisms including *Staph aureus Staph epidermidis Klebsiella Escherichia coli Enterococcus Pseudomonas β-haemol streptococcus* and *Strept viridans* The cerebrospinal fluid and the blood were sterile Decon tamination of the microflora was attempted by surface disinfection with Tego® (Goldschmidt) and administration of local antibiotics and non absorbable antibiotics includ ing fungistatics In addition carbenicillin oxacillin and gentamycin were administered intravenously As a result all bacteria were eliminated except Klebsiella which was suppressed but not completely eliminated After decon tamination at 19 days the patient was placed in a plastic isolation system designed and constructed in our hospital (6) He continued to receive antibiotics and fungistatics

Under these conditions the infant remained well until 15 weeks old when he weighed 3700 g He then suddenly deteriorated with a high intermittent fever up to 41 C and severe diarrhea Ronchi and inspiratory rales were heard over both lungs and there was marked inspiratory retrac tion Klebsiella was cultured from blood stool urine and

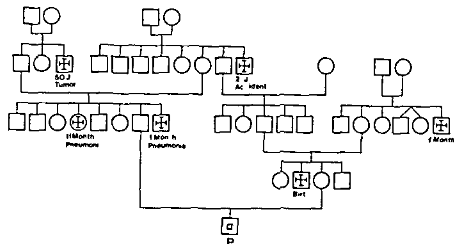


Fig 1 Pedigree of family V

in smears from the body orifices The patient died when he was 17 weeks old

Laboratory findings

General laboratory features Red cell counts Hb MCHC and reticulocytes were within normal range from birth until the 15th week During the last two weeks of life the child had septicaemic thrombocytopenia with lowest values in the order of 2×10^9/l and severe anaemia developed The white blood cell count was persistently below 1×10^9/l with marked lymphopenia Although most of the leucocytes were mononuclear cells some mature granulocytes were seen in the stained smears (Fig 2)

Total serum protein was around 60 g/l Normal values were found for sodium calcium potassium phosphorus chloride alkaline phosphatase and blood urea nitrogen levels Haemoglobins were normal with about 0 53 units HbF measured at birth

Bone marrow Smears of aspirated bone marrow were made when the boy was 6 and 12 weeks old Both smears *showed normal erythropoiesis and megakaryopoiesis* There were abundant promyelocytes but no distinct myelocytes metamyelocytes band or lobulated forms The number of reticular cells was high and many mast *cells were present*

Special investigations

Determination of serum factors The immunoglobulins were determined by a radial immuno-diffusion technique (9) using Partigen® plates (Behringwerke Marburg)

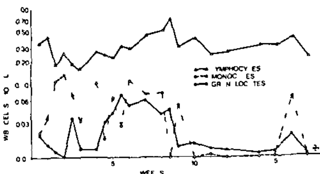


Fig 2 White blood cells $\times10^9$/l

Skin tests for delayed hypersensitivity The dinitrochlorobenzene test was performed according to the method of Aisemberg (1) A Candida skin test was done with Candida albicans extract 1 10 (Hollister Stier) administered in 0 1 ml

Lymphocyte cultures The in vitro response of lymphocytes to phytohemagglutinin (PHA) was tested with lymphocytes separated from heparinized blood using the method of Bøyum (4) 10^6 lymphocytes were cultured in 1 ml MEM S (Gibco) supplemented with 20% inactivated fetal calf serum 1% L glutamine and antibiotics PHA (Wellcome) was used at a final concentration of 1 125 The cells were harvested after 96 hrs

Mixed lymphocyte cultures were set up with $0\ 75\times10^6$ responder cells and $0\ 75\times10^6$x irradiated (2 000 rads) stimulator cells and harvested after 144 hrs at 37°C in 5% CO_2 0 06 μCi/mMol $2-{}^{14}C$ thymidine was added for the last 16 hrs of culture The uptake of the isotope was determined by liquid scintillation counting T-cells were detected by rosette formation with sheep erythrocytes *Lymphocytes were washed three times in gelatin veronal* buffer and resuspended in Hanks solution at a concentration of 2×10^6/ml The lymphocytes were incubated with sheep red blood cells for 15 minutes at 37°C and left overnight at 4 C (8)

Soft agar culture of bone marrow (BMC) and white blood cells (WBC) Heparinized bone marrow and peripheral leucocyte specimens obtained at the age of 12 weeks and before death were allowed to sediment at 1 G for 40 min at 4 C The supernatants were washed twice *with buffered balanced salt solution and the resulting cell* suspensions resuspended in culture medium (McCoy s 5A Gibco 0 3% Bacto Agar Difco) supplemented with 15% fetal calf serum

Plating of the hemopoietic cells was performed according to the procedure of Robinson et al (12) described in detail by Metcalf (10) All agar cultures were set up in triplicate and incubated in airtight glass containers at 37°C with 3% CO_2 and high humidity After 10 days incubation all aggregates of more than 50 cells were counted as colonies

Postmortem findings

The *thymus* consisted of a small number of fibrotic lobules No lymphocytes or Hasall s bodies were de

Table 1 *Evaluation of humoral immunity*

Age (weeks)	2	10	14
Serum Ig levels (mc/100 ml)			
G	859 0 (670–1 899)	164 0 (?34–1 751)	68 8 (159–708)
A	3 0 (0 ?2–2 87)	27 1 (1 7–71 8)	11 6 (6 8–60 7)
M	0 0 (7–59)	4 5 (??–61)	4 4 (?1–94)
E	–	0 01 (0 0?)[a]	0 03 (0 0?)[a]

Values in parenthesis refer to subjects 2 to 14 weeks of age
[a] *Normal adult values in our laboratory*

tected The weight was 1 g (normal 9–13 g) No *lymphnodes* were detected except for some very small nodes in the mesentery These were made up entirely of *reticular cells Lymphocytes and plasma cells were* absent The *spleen* was normal in size but without Malpighian follicles Reticular cells were predominant but a few scattered lymphocytes were found There was no evidence of extramedullary haemopoiesis In the gastrointestinal tract the *Peyer's patches were absent* Both *tonsils* were aplastic and showed no lymphocytes In the *lungs* most of the alveoli contained proteinaceous material and numerous bacteria but no polymorphonuclear cells Pneumocystis carinii could not be detected In the *liver* focal necroses haemosiderosis and bacteria were seen There was no evidence of inflammatory response around the necrotic areas No extramedullary haemopoiesis could be observed The *pericardium* contained proteinaceous material and numerous bacteria In the *myocardium* there were some non reactive necrotic areas and a subepicardial haematoma

Table 2 *Evaluation of cellular immunity and T lymphocytes*

	V V	Control
Circulating lymphocytes/10 /l	~0 5	>3 4
Delayed hypersensitivity (skin tests)		
Candida	Neg	Pos
DNCB	Neg	Pos
PHA response *Incorporation* of ¹⁴C-TdR		
c p m	169±13	13 381±1 630
Mixed leucocytes cultures c p m	V V +V V x 10±15	Contr +contr x 743±139
	V V +contr x ? 908±1 ?99	Contr +V V x 6 577±4 ?48
Stimulation index	13 84	8 86
Per cent of lymphocytes forming E rosettes	8	56

Stimulation index is the ratio of c p m of PHA stimulated cells to control cells

RESULTS

Immunological studies The results of the immunological studies are recorded in Tables 1 and 2 Serum immunoglobulins were decreased except for IgG levels recorded when the boy was 2 weeks old The percentage of peripheral blood lymphocytes was very low as is detailed in Fig 2 Data related to the thymus dependent system show that delayed hypersensitivity reactions were absent The in vitro lymphocyte response to PHA was markedly decreased when evaluated by thymidine incorporation The response of lymphocytes in mixed leucocyte culture was also reduced The percentage of lymphocytes forming E rosettes was very low The tests of cellular immunity were performed when the boy was 8–12 weeks old

Stem cell assay As shown in Table 3 the incidence of colony forming cells (CFCs) per 2×10^5 BMC of both the 2 year old and the healthy adult hematological patient was found to be within the normal range In addition the colony counts in WBC cultures of the control

Table 3 *Incidence of CFCs in the bone marrow and peripheral blood*

	No of CFCs per ?×10⁵ BMC	No of CFCs per 1×10 WBC
V V (1? weeks old)	16 (15–17)	No colonies
V V (preterminally)	No colonies	N D
Hematologically healthy controls		
(years old)	66 (63–70)	N D
(43 years-old)	54 (48–58)	1? (6–13)

were consistent with the usual findings obtained with healthy volunteers In the patient (V V) a substantial reduction of CFCs was found in the cultures at 12 weeks and no colony formation was observed in peripheral WBC cultures Immediately before death all cultures were negative All the colonies derived from the bone marrow specimen at 12 weeks were small in size (50–150 cells/colony) compared to the controls (50–2 000/colony) All aggregates consisted of granulocytic cells as far as could be determined by orcein staining

DISCUSSION

The histopathological findings in our patient were essentially the same as those described in the 4 known cases of the disease (2 7 15)

In our case the in vivo and in vitro tests of the cellular immunity were found to be decreased DNCB and candida skin test were negative and the response to phytohaemagglutinin stimulation was very low In the mixed leucocyte culture there was a weak response and the number of circulating lymphocytes was low (5) At postmortem depletion of lymphocytes in the lymphatic tissues and thymic aplasia were found The T cell defect was specifically proven by the low number of peripheral lymphocytes forming E rosettes Humoral immune response was also decreased as indicated by low serum Ig levels and the absence of plasma cells in the organs The IgG level found can be attributed to maternal IgG (16)

Experimental data (11) and a number of successful bone marrow transplantations in children with congenital combined immunodeficiencies suggest that the primary lesion in these patients is a stem cell defect (3) It has been shown that the pluripotent stem cell is not only the precursor in haemopoiesis but also supplies progenitors of the lymphatic cell series (13)

Our case is of particular interest with respect to the understanding of immunological diseases suspected to be caused by a stem cell defect since in addition to the combined immunodeficiency the patient suffered from agranulocytosis The in vitro colony forming cells (CFCs) are considered to be progenitors in granulopoiesis in the sense that they are committed stem cells As progeny of the pluripotent stem cells the CFCs are the most immature cells detectable in human hemopoietic cell suspension with the assay systems available It could be shown that the myelopoietic progenitor cell content (CFCs) was diminished in the patient s bone marrow when the infant was 12 weeks old and absent before death

The following possible explanations for this finding can be considered

(*a*) There may be a genetic defect of pluripotent stem cells with regard to differentiation in myelopoiesis or lymphopoiesis

(*b*) Microenvironmental factors could inhibit the differentiation of stem cells in myelopoiesis (14)

However as the microenvironmental factors in myelopoiesis are probably related to interactions between adjacent bone marrow stroma cells and as our test system was done in vitro, it is unlikely that the latter mechanism plays a role in these disorders

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Submitted June 21 1976
Accepted Oct 27 1976

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Acta Pædiatr Scand 66 285–287 1977

CORD BLOOD PORPHYRINS

A Quantitative Assay in Newborn Infants

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ABSTRACT Handa F and Sharma S C (Department of Dermato-Venereology Government Medical College and Rajendra Hospital Patiala Punjab India) Cord blood porphyrins A quantitative assay in newborn babies Acta Paediatr Scand 66 285 1977 —A quantitative assay of free erythrocyte porphyrins was done in umbilical cord blood of 30 newborn babies by a spectrophotometric method The mean value of free erythrocyte protoporphyrin in umbilical cord blood was found to be 74.58 μg/100 ml packed erythrocytes and that of free erythrocyte coproporphyrin to be 0 77 μg/100 ml packed erythrocytes with a standard deviation of ±29 99 and ±1 48 respectively Relationship between free erythrocyte protoporphyrin and free erythrocyte coproporphyrin and their correlations with birth weight and sex of the newborn baby were found to be statistically significant

KEY WORDS Cord blood porphyrins free erythrocyte protoporphyrin free erythrocyte coproporphyrin newborns

Since the first detection of free erythrocyte porphyrin by Hijmans van den Bergh and Hyman in 1928 number of workers in various parts of the world have done quantitative estimation of free erythrocyte porphyrins in adults using different techniques Only few workers like Schwartz & Wikoff (4) Hsia & Page (1) Prato et al (2) Ventura & Meduri (5) and Wranne (6) have done quantitative estimation of free erythrocyte protoporphyrin (EPP) and free erythrocyte coproporphyrin (ECP) in umbilical cord blood No contribution from this part of the world has been made Hence the present study was carried out to establish normal levels of free erythrocyte porphyrins in umbilical cord blood of Indian newborn babies

MATERIAL AND METHODS

Rimington s (3) sensitive and reproducible technique based on the Broadsheet 70 (Revised Broadsheet 36) 1971 was used for the present study Umbilical cord blood samples were obtained from 30 newborn babies delivered in the Obstetric Department of this hospital Labour had not been difficult and asphyxia congenital anomaly disease or birth injury had not been observed in these babies Birth weights ranged from 000–3400 g with a mean of 2550 g

Deliveries occurred between 10 days before to 16 days after the expected date of delivery Haemoglobin in mothers ranged from 8 0–11 5 g/100 ml with a mean of 9 5 g/100 ml Age of the mothers was between 17 years and 35 years with a mean age of 24 5 years Parity of the mothers ranged from 1st–8th para with 3rd para as the mean parity Besides there was no Rh incompatibility between the mother and the newborn baby and no complication or medication (except supportive iron and vitamin therapy) during pregnancy

RESULTS

Normal levels of cord blood porphyrins

The mean EPP level was 74 58 μg/100 ml packed erythrocytes with a range of 33 10–141 22 μg/100 ml and mean ECP level was 0 77 μg/100 ml with a range of 0 00–4 49

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ABSTRACT Handa F and Sharma S C (Department of Dermato-Venereology Government Medical College and Rajendra Hospital Patiala Punjab India) Cord blood porphyrins A quantitative assay in newborn babies. Acta Paediatr Scand 66 285 1977.—A quantitative assay of free erythrocyte porphyrins was done in umbilical cord blood of 30 newborn babies by a spectrophotometric method The mean value of free erythrocyte protoporphyrin in umbilical cord blood was found to be 74.58 μg/100 ml packed erythrocytes and that of free erythrocyte coproporphyrin to be 0 77 μg/100 ml packed erythrocytes with a standard deviation of ±29 99 and ±1 48 respectively Relationship between free erythrocyte protoporphyrin and free erythrocyte coproporphyrin and their correlations with birth weight and sex of the newborn baby were found to be statistically significant

KEY WORDS Cord blood porphyrins free erythrocyte protoporphyrin free erythrocyte coproporphyrin newborns

Since the first detection of free erythrocyte porphyrin by Hijmans van den Bergh and Hyman in 1928 number of workers in various parts of the world have done quantitative estimation of free erythrocyte porphyrins in adults using different techniques Only few workers like Schwartz & Wikoff (4) Hsia & Page (1) Prato et al (2) Ventura & Meduri (5) and Wranne (6) have done quantitative estimation of free erythrocyte protoporphyrin (EPP) and free erythrocyte coproporphyrin (ECP) in umbilical cord blood No contribution from this part of the world has been made Hence the present study was carried out to establish normal levels of free erythrocyte porphyrins in umbilical cord blood of Indian newborn babies

MATERIAL AND METHODS

Rimington's (3) sensitive and reproducible technique based on the Broadsheet 70 (Revised Broadsheet 36) 1971 was used for the present study Umbilical cord blood samples were obtained from 30 newborn babies delivered in the Obstetric Department of this hospital Labour had not been difficult and asphyxia congenital anomaly disease or birth injury had not been observed in these babies Birth weights ranged from 2000–3400 g with a mean of 2550 g

Deliveries occurred between 10 days before to 16 days after the expected date of delivery Haemoglobin in mothers ranged from 8 0–11 5 g/100 ml with a mean of 9 5 g/100 ml Age of the mothers was between 17 years and 35 years with a mean age of 24 5 years Parity of the mothers ranged from 1st–8th para with 3rd para as the mean parity Besides there was no Rh incompatibility between the mother and the newborn baby and no complication or medication (except supportive iron and vitamin therapy) during pregnancy

RESULTS

Normal levels of cord blood porphyrins

The mean EPP level was 74 58 μg/100 ml packed erythrocytes with a range of 33 10–141 22 μg/100 ml and mean ECP level was 0 77 μg/100 ml with a range of 0 00–4 49

19 77 8 3

bilical cord blood obtained by various workers This can partly be explained by differences in the analytical procedures Racial differences could also add to this variation

ACKNOWLEDGEMENTS

We are thankful to Mr V S Joshi Lecturer in Statistics for his help and guidance in the statistical analysis of this study

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Submitted June 18 1976
Accepted Nov 8 1976

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Table 1 *Levels of cord blood porphyrins in various studies*

Study no	Authors	No of cases	ECP (μg/100 ml)		EPP (μg/100 ml)	
			Mean	*Range*	*Mean*	*Range*
1	Schwartz & Wikoff (1952)	2	–	8 1 5 3	–	178 69
2	Hsia & Page (1954)	6	1 5	0 6–2 8	125	63–203
3	Prato Mazza & Fiorina (1959)	10	7 9	4 4–12 6	72	46–116
4	Wranne (1960)[b]	20	2 8	1 1–7 2	54	32–135
5	*Present study (1976)*	30	0 77	0 00–4 49	74 58	33 10–141 22

Schwartz & Wikoff method of 1952 used and plasma included
[b] *Coproporphyrin included traces of protoporphyrin*

μg/100ml The highest EPP value of 141 22 μg/100 ml corresponded to the highest value of ECP 4 49 μg/100 ml and the second highest value of EPP 132 41 μg/100 ml also corresponded to the second highest value of ECP 4 09 μg/100 ml Correlation between EPP and ECP was found to be positive and statistically *significant (t value was 7 84 more than 2 05* table value t for 28 D F at $p=0$ 05)

Thus, increase in the levels of EPP corresponded to an increase in the levels of ECP

Correlation with sex of newborn baby

Protoporphyrin Mean level of EPP of 93 90 μg/100 ml with a range of 42 05–141 22 μg/100 ml in male babies more than the mean level of *EPP of 63 39 μg/100 ml with a range of 33 10–124 13 μg/100 ml in female babies and* this difference in the mean levels was statistically significant (t value was 2 59)

Coproporphyrin Mean level of ECP of 1 61 μg/100 ml with a range of 0 00–0 49 μg/100 ml in male babies was higher than the mean level of ECP of 0 28 μg/100 ml with a range of 0 00–2 96 μg/100 ml in female babies and this difference in the mean value was statistically significant (t value was 2 05)

Thus, the levels of EPP and ECP were higher in male as compared to female newborn babies

Correlation with birth weight

Protoporphyrin Correlation between EPP and birth weight was positive and statistically significant (t value was 7 05)

Coproporphyrin Correlation between EC and birth weight was positive and statistical significant (t value was 5 84)

DISCUSSION

In our study the highest and the second highe *EPP values correspond to the highest and th* second highest ECP values These findings ar similar to the findings of Wranne (6) But i addition we could establish a positive ar statistically significant correlation betwee EPP and ECP which is in contrast to the find ings of Wranne (6) who could not establis any such correlation Thus increase in th levels of EPP are accompanied by a co *responding increase in the levels of ECP in th umbilical cord blood*

In the present study we could also establis that values of EPP and ECP are significantl higher in the male than in the female newborn A possible explanation for this could be tha on an average the boys had a higher birt weight than the girls in our study

We could also establish a positive an significant correlation between the EPP an ECP values and the birth weight With highe birth weight there is a corresponding increas in the levels of EPP and ECP This may partl *be explained by an increased erythropoesi* caused by hypoxia in the heavier babies

Levels of EPP and ECP obtained in th present study are compared with the finding of earlier workers (Table 1) There is a wid *variation in the levels of EPP and ECP in um*

Acta Pædiatr Scand 66 289–295 1977

GENERAL HEALTH SCREENING OF FOUR YEAR OLDS IN A SWEDISH COUNTY

V A Strategy for Improving the Effectiveness and the Costefficiency of the Psychological Screening Program

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From the Department of Paediatrics University Hospital Uppsala Sweden and the Institute of Social and Preventive Medicine University of Berne Berne Switzerland

ABSTRACT Nilsson C Sundelin C and Vuille J-C (Department of Paediatrics, University Hospital Uppsala Sweden and Institute of Social and Preventive Medicine University of Berne Berne Switzerland) General health screening of four year-olds in a Swedish county V A strategy for improving the effectiveness and the cost-efficiency of the psychological screening program Acta Paediatr Scand 66 289 1977.—Data presented in a previous paper pointed to the necessity for improving the overall sensitivity of the psychological screening program The present report indicates possibilities for such an improvement without changing the screening methods A comparison of the primary data of the true positives the false positives and all the negatives (non referred) revealed the necessity of more stringent referral criteria It is predicted that the systematic application of these criteria would result in an increase in the rate of true positives form 2 8 to 4 8% of the screened population In addition a strategy aiming at a reduction of the costs without deteriorating the effectiveness and based on a differential application of the various elements of the screening program is presented

KEY WORDS Pre school children psychological screening effectiveness cost-efficiency

In a previous report it was shown that the sensitivity of the program used for psychological screening within the context of a routine health examination of four year olds was not optimal (5) on the other hand the specificity and the positive predictive value as additional measures of effectiveness were considered to be acceptable (For definitions see ref 5) Two possibilities for improving the sensitivity were outlined

(*a*) Development of completely new instruments This would necessitate an assessment not only of the validity of the new methods in an experimental situation but also of their performance under the practical conditions of the field work Such a development may be extremely time consuming and it must be acknowledged that it might not bring about any improvement at all

(*b*) The yield of the methods now in use might be increased if more rigorous criteria could be established for the identification of those children who would benefit from referral to a specialist In the present report an attempt is made to define a number of such criteria on the basis of an analysis of the primary data of referred and non referred children

In addition a strategy allowing a reduction of the costs without deteriorating the results will be presented

METHODS

The methods used for screening and for the clinical assessment have been described previously (5) In essence

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Table 1 *Definition of scores 0 1 and 2 in the four screening instruments*

Instrument	Score 0	1	2
Questionnaire	No problems according to the parents	Somewhat late in development and/or mild behaviour problems and/or latent need of help	Retarded development and/or severe behaviour problems and/or manifest need of help
Interview (sum of 27 items each item being coded 0 1 or 2)	0–3	4–9	10+
Examination/observation (sum of 5 items each item being coded 0 1 or 2)	0	1–5	6+
Physician s final rating	Normal for age	Somewhat deviant	Definitely deviant

the screening procedure was based on four different sources of data

(1) A simple questionnaire completed by the parents
(2) an interview conducted by the responsible nurse
(3) an examination of the child s development and direct observation of his behavior and
(4) an overall rating by the physician

Each source of data yielded a varying number of items In order to simplify the analysis the entire information from each method was reduced to a single score in the scale 0 1 2 according to the criteria shown in Table 1 The sum of the four scores will be called the total score

As a result of the clinical assessment each referred child was assigned to one of three categories healthy child at risk and definitely deviant An additional classification was made in the group of referred children according to the *main symptom* aggressiveness anxiety infantile symptoms (enuresis encopresis thumb sucking) restlessness/tension (hyperactivity sleep disturbance problems of contact tics nail-biting) The assignment to one of these classes was based on the number and the severity of the individual symptoms within each category as well as on the general prognostic significance of the symptoms (determined by common agreement of a group of child psychiatrists (4))

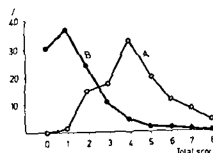


Fig 1 Percentual distribution of referred (open circles) and non referred (closed circles) children with respect to total score

MATERIAL

The present analysis is based on data concerning the children examined in 1969 and 1970 Of the 3 897 invited children 3 810 participated in the screening (=97 7%) For the present purpose only those with complete primary data are included The numbers may therefore vary somewhat between the different analyses depending on the variable(s) under study

Group A Children referred to the special assessment unit Originally this group comprised 158 children but only 127 of these were eventually seen by a specialist (5)

Subgroup A_T Referred children considered to be in need of treatment (child at risk and definitely deviant) $n=85$

Subgroup A_H Referred children declared healthy by the specialist $n=42$

Group B Non referred children with complete data $N=3\,008$

RESULTS AND DISCUSSION

1 *Total score in referred and non referred children*

In Fig 1 the distributions of the total score in groups *A* and *B* show a significant overlap This indicates that the decision as to whether or not a child should be referred to a specialist did not depend exclusively on the total number of problems symptoms or failures emerging in the test but rather *on the result of an overall* interpretation of the possible significance of these findings The details of this process are largely unknown but some special features may be elucidated from the available data

The following calculations were made under

Table 2 *Distribution of screening scores in referred children*

A_T = in need of treatment A_H healthy

Screening instrument	Clinical category	Screening scores (percentage distribution) 0	1	2	χ^2
Questionnaire	A_T n=7[illegible]	24.0	37.3	38.7	[illegible].85 *n s*
	A_H n=36	41.7	25.0	33.3	
Interview	A_T n=85	12.9	41.2	45.9	1[illegible].88
	A_H n=4[illegible]	40.5	33.3	6[illegible]	p<0.01
Examination & observation	A_T n=83	14.5	60.[illegible]	25.3	[illegible].43 *n s*
	A_H n=38	7.9	81.6	10.5	
Physician's rating	A_T n=84	39.3	29.8	30.9	0.36 *n s*
	A_H n=40	37.5	35.0	27.5	

the assumption that if additional children had been referred to the specialists because their data fulfilled certain criteria they would have been assigned to subgroups A_T and A_H in the same proportions as those children who had been referred in reality The observation (5) that a group of preschool children referred to the specialist team through mechanisms other than screening was composed of healthy at risk and deviant children in proportions equal to those of group A shows that this assumption is probably realistic

2 *Total score in subgroups A_H and A_T*

Fig 2 depicts a considerable overlap This is an important finding because it indicates that the total score alone is not a very useful measure for the precise identification of children who will eventually need treatment In the region of score 5 or more however the discrimination appears satisfactory Thus if all children who had accumulated scores of 5 to 8 had been included another 48 referrals would have resulted Under the assumption mentioned above 32 of these might have been justified and 16 unnecessary

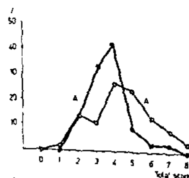


Fig 2 Referred children Percentual distribution of subgroups A_T (in need of treatment open circles) and A_H (healthy closed circles) with respect to total score at screening

3 *The discriminating power of the four screening instruments*

In Table 2 subgroups A_T and A_H are compared with respect to the relative proportions of children with scores 0 1 and 2 in each of the four sets of data A significant difference (tested by means of χ^2) is evident only for the interview score A similar though not significant tendency is noted with respect to the questionnaire and the direct examination score whereas the physician's final rating does not discriminate at all

The interview's discriminating power could be utilized in the following way If—in addition to those already screened out by the total score criterion—all children with an interview score of 2 had been referred to a specialist an additional 45 children probably needing treatment would have been assessed but at the same time the specialist team would have had to examine another 22 healthy children

If combined the criteria discussed in paragraphs 2 and 3 would have given an increase in the rate of correct referrals (children really in

Table I *Definition of scores 0 1 and 2 in the four screening instruments*

Instrument	Score 0	1	2
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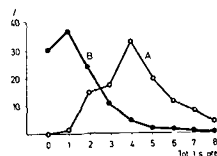


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	A_H n=36	41 7	25 0	33 3	
Interview	A_T n=85	1? 9	41 2	45 9	1? 88
	A_H n=4?	40.5	33 3	6 2	$p<0$ 01
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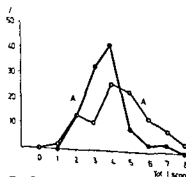


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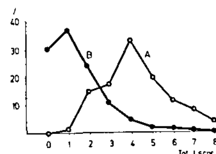


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The following calculations were made under

Table 4 *Conducted interviews in per cent of participants and rate of referrals for psychological problems*

	1969	1970	Change of instructions ↓	1971
Conducted interviews %				
Rural districts	98	100		91
Urban districts	96	97		65
Rural+urban	96	98		77
Referrals %	5 4	3 7		5 0

whereas the simple developmental examination could be performed rapidly The observation of the child s behaviour was also an inexpensive method since it was made at the same time as the various examinations (physical and other) As a rule the completion of the questionnaire did not demand the participation of a professional

We therefore determined that percentage of the finally referred children for whom the questionnaire indicated a severe or a slight problem to these were added children with scores of 2 or 1 in the direct observation and examination The corresponding percentages for the total population of screened children were also computed

The result presented in Fig 3 shows that a combination of the data obtained from the questionnaire and from the direct observation and examination of the child could have identified 97% of all children who were finally referred It seems as if the interview data were used primarily for a further differentiation among those in whom the other sources had already pointed to the existence of some problems This observation prompted a change in strategy at the end of the year 1970 Thus according to the new instructions the interview should be carried out only in those cases where the other data had revealed a problem and in all previously unknown children and all previously known problem children The reason why the interview was not abolished completely was the finding that this instrument had a high discriminating power between correct and unnecessary referrals (see p 291) Although it did not add very much to the overall sensitivity of the screening program it constituted an important means of assuring a sufficiently high specificity It was anticipated that about 1/2 to 2/3 of the time spent on interviews could be saved by this strategy (Fig 3)

Table 4 shows what happened in reality the nurses especially those in the rural districts continued to conduct the interviews in the majority of cases probably because they had learned to regard this instrument as a means of establishing contact with the families in their district Nevertheless there was a significant decrease in the overall rate of conducted interviews As expected this decrease did not affect the rate of referrals for psychological problems Further the positive predictive value of the screening process remained essentially unchanged (71% as opposed to 67% in 1969 and 1970)

CONCLUSIONS

This analysis has shown that the screening procedure can be rationalized by a differentiated application of the various instruments without reducing the effectiveness The sensitivity which is the most important parameter of effectiveness could be improved considerably if all children with an overall interview score of 2, those with a total score of 5 or more and those with a heavy symptom load in the restlessness/tension complex were referred for specialist examination The anticipated gain in sensitivity (increase of correct referrals from 2 8 to 4 8% of all children) is probably a conservative estimate since it is based on the assumption that the proportion of false positives among the additional referrals would be the same as in those referred for the usual reasons As a matter of fact the additional referral criteria proposed here were chosen just because of their especially high discriminating power and the proportion of

Table 3 *Distribution of referred children with respect to main symptoms*

Main symptom	Classification by the specialist			
	Healthy (n=42)		Definitely deviant (n=27)	
	No	%	No	%
Aggressiveness	7	17	3	11
Anxiety	19	45	5	19
Infantile behaviour	4	10	1	4
Restlessness/ tension	7	17	18	67
No symptoms	5	12	0	0

$\chi^2=17\ 11$ d f =4 $p<0\ 005$

need of treatment) from 2 8 to 4 8% of the total population or from 2 2 to 3 8% if allowance is made for those 20% of the referred who would refrain from the proposed special consultation This theoretical result could have been achieved without any change in the screening procedure but probably at the cost of specialist examinations of another 30 healthy children

4 *Qualitative aspects of the criteria for referral*

The percentual distribution of the children in group *A* with respect to the main symptoms is shown in Table 3 There is a significant difference between subgroups A_H and A_T the children needing treatment presenting more often with symptoms within the class restlessness/ tension whereas anxiety dominated as the main symptom in those declared to be healthy A similar classification was made in groups *A* and *B* on the basis of the symptoms elicited in the screening interview

No significant difference was found between the two groups with respect to the pattern of symptoms though of course all the symptoms occurred more frequently in group *A* This probably indicates that the decision for referral was not based on the qualitative aspects—at least not in a systematic manner In view of the specialists more serious rating of the restlessness/tension complex however these symptoms should be given particular weight as a referral criterion

5 *Improving the cost efficiency*

The systematic application of the criteria mentioned in paragraphs 2 3 and 4 might be expected to improve the effectiveness of the screening process The costs of the screening would remain unchanged but the work load at the assessment unit would increase significantly Therefore this modification of the strategy would probably not change the overall cost efficiency ratio Since the total cost is an important issue in view of the limited resources available attempts were made early in the process of program evaluation to diminish the costs without changing the effectiveness (8)

The most expensive item in terms of manpower was the interview with the parents

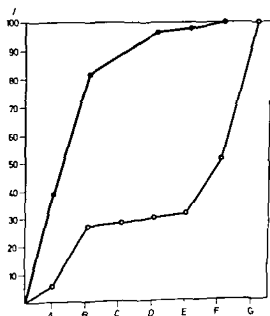


Fig 3 Cumulative percentage of children in referred group (closed circles) and in total material (open circles) with scores of 2 and 1 in questionnaire examination/observation and in interview Instruments ordered according to their relative costs (inexpensive→expensive)

A Questionnaire score 2
B +questionnaire score 1
C +exam /observation score 2
D +exam /observation score 1
E +interview score 2
F +interview score 1
G +those without indication of problems

Table 4 *Conducted interviews in per cent of participants and rate of referrals for psycho logical problems*

	1969	1970	Change of in struc tions ↓	1971
Conducted interviews %				
Rural districts	98	100		91
Urban districts	96	97		65
Rural+urban	96	98		77
Referrals %	5 4	3 7		5 0

whereas the simple developmental examina tion could be performed rapidly The observa tion of the child s behaviour was also an in expensive method since it was made at the same time as the various examinations (physi cal and other) As a rule the completion of the questionnaire did not demand the participation of a professional

We therefore determined that percentage of the finally referred children for whom the questionnaire indicated a severe or a slight problem to these were added children with scores of 2 or 1 in the direct observation and examination The corresponding percentages for the total population of screened children were also computed

The result presented in Fig 3 shows that a combination of the data obtained from the questionnaire and from the direct observation and examination of the child could have identi fied 97% of all children who were finally re ferred It seems as if the interview data were used primarily for a further differentiation among those in whom the other sources had already pointed to the existence of some prob lems This observation prompted a change in strategy at the end of the year 1970 Thus ac cording to the new instructions the interview should be carried out only in those cases where the other data had revealed a problem and in all previously unknown children and all previously known problem children The reason why the interview was not abolished completely was the finding that this instrument had a high discriminating power between cor rect and unnecessary referrals (see p 291) Although it did not add very much to the over all sensitivity of the screening program it con stituted an important means of assuring a suf ficiently high specificity It was anticipated that about 1/2 to 2/3 of the time spent on inter views could be saved by this strategy (Fig 3)

Table 4 shows what happened in reality the nurses especially those in the rural districts continued to conduct the interviews in the majority of cases probably because they had learned to regard this instrument as a means of establishing contact with the families in their district Nevertheless there was a significant decrease in the overall rate of conducted inter views As expected this decrease did not af fect the rate of referrals for psychological problems Further the positive predictive value of the screening process remained es sentially unchanged (71% as opposed to 67% in 1969 and 1970)

CONCLUSIONS

This analysis has shown that the screening procedure can be rationalized by a differenti ated application of the various instruments without reducing the effectiveness The sensi tivity which is the most important parameter of effectiveness could be improved consider ably if all children with an overall interview score of 2 those with a total score of 5 or more and those with a heavy symptom load in the restlessness/tension complex were re ferred for specialist examination The anti cipated gain in sensitivity (increase of correct referrals from 2 8 to 4 8% of all children) is probably a conservative estimate since it is based on the assumption that the proportion of false positives among the additional referrals would be the same as in those referred for the usual reasons As a matter of fact the addi tional referral criteria proposed here were chosen just because of their especially high discriminating power and the proportion of

Table 3 *Distribution of referred children with respect to main symptoms*

Main symptom	Classification by the specialist: Healthy (n=42) No	%	Definitely deviant (n=27) No	%
Aggressiveness	7	17	3	11
Anxiety	19	45	5	19
Infantile behaviour	4	10	1	4
Restlessness/tension	7	17	18	67
No symptoms	5	12	0	0

χ^2=17 11 d f =4 p<0 005

need of treatment) from 2 8 to 4 8% of the total population or from 2 2 to 3 8% if allowance is made for those 20% of the referred who would refrain from the proposed special consultation This theoretical result could have been achieved without any change in the screening procedure but probably at the cost of specialist examinations of another 30 healthy children

4 *Qualitative aspects of the criteria for referral*

The percentual distribution of the children in group A with respect to the main symptoms is shown in Table 3 There is a significant difference between subgroups A_H and A_T the children needing treatment presenting more often with symptoms within the class restlessness/tension whereas anxiety dominated as the main symptom in those declared to be healthy A similar classification was made in groups A and B on the basis of the symptoms elicited in the screening interview

No significant difference was found between the two groups with respect to the pattern of symptoms though of course all the symptoms occurred more frequently in group A This probably indicates that the decision for referral was *not* based on the qualitative aspects—at least not in a systematic manner In view of the specialists more serious rating of the restlessness/tension complex however these symptoms should be given particular weight as a referral criterion

5 *Improving the cost efficiency*

The systematic application of the criteria mentioned in paragraphs 2 3 and 4 might be expected to improve the effectiveness of the screening process The costs of the screening would remain unchanged but the work load at the assessment unit would increase significantly Therefore this modification of the strategy would probably not change the overall cost efficiency ratio Since the total cost is an important issue in view of the limited resources available attempts were made early in the process of program evaluation to diminish the costs without changing the effectiveness (8)

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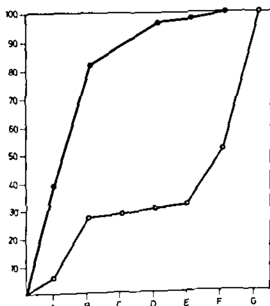


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Submitted May 21 1976
Accepted Nov 3 1976

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false positive might therefore be even lower The implementation of these criteria would imply more clerical work for the staff since the scores have to be computed from a number of items Therefore if an automatic monitoring system is available (7) these criteria could be built in so that those cases who were not referred in spite of fulfilment of the criteria would be revealed and discussed after the screening

If the present procedure of performing the interview only in selected cases is retained the possible gain in sensitivity will be somewhat lower than the theoretical figure anticipated in paragraph 3 but it may still be expected to be substantial

The conclusions in this article rely heavily on the validity of the specialists evaluation of the referred children s need of treatment The relatively serious prognosis attached to the hyperactivity syndrome is in accordance with published experience (2 6) but as yet we do not know whether the problems of those children who have been declared healthy really were trivial and self limiting Furthermore certain deviating behaviours though troublesome for parents and others may very well be helpful for the child himself We believe however that it is important to identify not only disturbed children but also those parents who need help in order to arrive at a true understanding of their troublesome child The emphasis on the child s symptoms during the screening appears to be the most effective way to enable parents to engage in a discussion with specialists concerning their attitude towards the child We are not able to prove however that this aim has been achieved A systematic follow up now in progress may provide an answer to this and other questions raised in this paragraph

Systematic screening of preschool children for psychological problems is a relatively new enterprise and there are very few reports with which our results can be compared Prevalence rates from epidemiological studies without therapeutic intervention (1 3) should be compared with frequencies of symptoms or of combined scores as revealed by our screening instruments rather than with the percentage of children finally receiving some treatment In our view, however, this latter figure is the only relevant one if we accept the assumption that the treatment has a beneficial influence on the future development of the child At the present time no reliable data are available concerning the number of children who might really profit from some sort of intervention The true figure may be very much higher than the few per cent found in our investigation In view of the many uncertainties in this field however it would appear wise to study the experience gained from a restricted group before any drastic expansion of the resources for intervention is considered

In this context it is necessary to point out that some counselling was also provided for the non referred children at the Child Health Centers Unfortunately we have no quantitative data on this unsophisticated therapeutic activity but we know from personal experience that it represented a positive complement to the referral routines especially for those parents who hesitated to consult a psychologist or a psychiatrist We believe in fact that an optimal model for preventive action will have to integrate a number of activities guided by laymen as well as by professionals of different levels of formal competence and from various specialties Screening and subsequent treatment by specialists represents one important part of a comprehensive program and it may very well serve as a starting point for further developments

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PHYSICAL HEALTH OF 7 YEAR OLD CHILDREN

An Epidemiological Study of School Entrants and a Comparison with Their Preschool Health

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ABSTRACT Kohler L (Department of Paediatrics University Hospital Lund and Dalby Community Health Research Centre Dalby Sweden) Physical health of 7 year-old children An epidemiological study of school entrants and a comparison with preschool health Acta Paediatr Scand 66 297 1977.—At 7 years of age all 649 7 year-old children in a school district underwent a physical examination a vision screening and an auditory screening 210 of the children were previously examined in an extensive health control at 4 years of age The purpose of the present study was to describe the children s health situation and to evaluate the special health control performed at 4 years of age In 15% of the children functionally important health problems were found Visual defects were most common comprising 7.5% then came physical health problems such as motor disturbances obesity bacteriuria in 6 5% and hearing defects in 1% About half of the important health problems were previously known Children who had passed the special health control at 4 years of age had fewer newly detected important health problems and more previously known ones than other children which means that many children with above all visual defects but also motor disturbances bacteriuria and testis retention were detected and treated earlier than would have happened without the special control at 4 years It is concluded that the ordinary preschool Child Health Services did fulfill their purpose to detect handicapping disorders in an acceptable way by the introduction of the special health control at 4 years of age this function was further improved

KEY WORDS School health vision screening hearing screening obesity

The aim of the school health programme is to keep and increase the children s physical and mental health and to prevent and detect disease and handicap The modern school with long compulsory education and new curricula has also influenced the extense and intension of school health services Thus to the physical health examination formerly the one and only form of health surveillance have been added aspects of the children s mental health and social welfare this means that several new groups of personnel have been included in the health service beside physicians and nurses e g psychologists and social workers However the doctor and his physical examinations still play an important role in the school health programme

One of the purposes with the present study was to describe in school entrants the frequency and types of physical disabilities that might influence the present and future function of the child

Another purpose was to evaluate a special health control of four year-old children which was introduced some years earlier (14) Already at the planning of this examination of preschool children it was decided that it should be repeated when the children began school at the age of seven In addition another group of 7 year-old children who had not been

Table 2 *Physical examination*

Health problems	Not examined at 4 years $n=439$ (category not 4)		Examined at 4 years $n=210$ (category 4)		Functionally important health problems at 4 years	Sum $n=649$	
	n	%	n	%	n	n	%
(a) Motor disturbance group 1	10	2.3	13	6.2	0	23	3.5
(b) Motor disturbance group 2	4	0.9	3	1.4	3	7	1.1
(c) Motor disturbance group 3	1	0.2	0	0	0	1	0.2
Total %		3.4		7.6			4.8
(d) Obesity >+3 S D	15	3.4	10	4.8	3	25	3.9
(e) Bacteriuria	3	1.4	0	0	0	3	0.9
(f) Multiple malformations	0	0	2	0.9	2	2	0.3
(g) Retentio testis	3	1.3*	0	0	0	3	0.9
(h) Phimosis	0	0	2	0.9	0	2	0.3
(i) Inguinal hernia	2	0.5	0	0	0	2	0.3
(k) Adenoid	1	0.2	0	0	0	1	0.2
(l) Organic heart disease	1	0.2	0	0	0	1	0.2
(m) Flat feet	1	0.2	2	0.9	0		
Sum functionally important health problems (all in b c d e f g l)	27	6.1	15	7.1	8	42	6.5
Newly detected (in b d e g)	15	3.4	7	3.3		22	3.4

% of the girls
* of the boys

A level of 20 dB ISO was considered normal except at the lower frequences (250 and 500 cps) where 25 dB was accepted because of the unavoidable enviromental noise in the testing room

Children with hearing impairment at [illegible] or more frequences on the same ear were referred to the audiologist for further evaluation According to this professional evaluation a classification of the hearing impairments was made (Table 1)

Children with health problems considered to be functionally important at the present or in the future and not under current professional care were referred for further evaluation via the ordinary school physician After evaluation and diagnosis the health problems were graded according to their importance for the children s health and development (13 14) (Table 1)

Groups 2 and 3 were considered as *functionally important health problems* corresponding to the same definition at the health control of 4-year-olds (11 12 13 14) *Previously known* means that the health problems had already been detected and cared for before the actual investigation

RESULTS

Deviations revealed in the physical examination are presented in Table 2 Functionally important health problems in these children at 4 years of age are also shown

Motor disturbances dominate the picture just as they did at 4 years of age In category not 4 2 3% and in category 4 6 2% had motor disturbances The majority though showed only slight deviations i e immature clumsy gross or fine movements or slight tremor The difference of frequency between the two categories is not statistically significant ($p>0.05$) The more serious deviations (groups 2 and 3) concerned a boy with an operated hydrocephalus a boy with an operated cerebellar tumour 2 boys and 1 girl with brain damage and epilepsy and 3 children with pronounced motor difficulties combined with hyperactivity and short attention span (minimal brain dysfunction MBD) Seven of these children were previously known and treated Sixteen children were investigated when they were 4 years old only 3 had then functionally important deviations (the boy with hydrocephalus and 2 boys with MBD)

Overweight was also frequent in 59 children (9 1%) the weight was 2 S D above their standard weight for height and in 25 children

Table 1 *Classification of health problems*

Method of examination	Group 0 Healthy child	Group 1 Slight deviation without importance	*Group 2* Moderate deviation *Treatment indicated*	*Group 3* Definitely handicapping disorder
General physical examination		Flat feet phimosis	Retentio testis MBD obesity	Cerebral palsy organic heart disease
Vision examination		*Myopia*<−*1* 0 D Hyperopia<+1 5 D Astigmatism<±1 5 D *Slight* heterophoria	Refractive errors without amblyopia Heterophoria with complaints	Amblyopia strabismus
Auditory examination		Mild otosalpingitis cured by simple otological measures and few visits to the physician	Protracted otosalpingitis requiring more intense therapy (adenoidectomy drainage tubes) Moderate sensorineural hearing impairments with need for follow up and control	Severe impairment requiring hearing aid or special education

investigated in this way earlier were examined Thus it should be possible to compare the physical health of a group of 7 year old children with the health of the same children at 4 years of age as well as with another group of 7 year old children who had not passed the special health control at the age of 4 years

MATERIAL

In the first class at the age of 7 years 649 children born 1962–1965 and living in the school district of Dalby in Southern Sweden were examined

The school district is congruent with the medical district where the special health examinations of 4 year-old children were performed in 1968–1969 (14) Out of the 649 school entrants 210 were examined as preschool children (called category 4) and 439 were not examined as preschool children (called category not 4)

METHODS

General physical examination

The physical examination consisted of a somewhat extended class examination according to a structured and standardised form It was performed in the schools by the author assisted by a registered nurse By a careful inspection and palpation of the undressed child abnormalities of the mouth throat superficial lymph nodes thyroid gland skin skeleton muscles and abdomen were recorded In boys the testes and the foreskin were examined Heart and lungs were auscultated pulsations of the femoral arteries were palpated

Posture and spontaneous motor behaviour were observed with the child sitting standing walking and running Attention was paid to asymmetry in stature and movements and also to the presence of involuntary movements Further observation of the gross movements and coordination was made while the child was walking along a straight line walking on tiptoe and on the heels and standing or hopping on one leg Fine movements and coordination were studied while the child was cutting a piece of paper with a pair of scissors and was threading small wooden beads on a string

Inappropriate activity and disordered attention span in a child during the examination were also recorded e g constantly moving around touching and handling objects without discernible purpose Screening for bacteriuria was performed by a test paper Uriglox* which in previous studies in preschool and school children was found to be very reliable (24) Height and weight were measured with the school s standard equipment a scale on the wall with a wooden headpiece and a platform balance Weight for height was calculated on a standard curve for Swedish children (2)

Vision examination

The monocular visual acuity was tested by the school nurse with a linear E-chart (Oculus) at a distance of 5 meters Children with a visual acuity of 0 9 or less on one or both eyes were referred to the ophthalmologist for further evaluation (12) After the professional examination the visual deviations were classified according to their impact on the children s health and function and on the need for care (Table 1)

Auditory examination

The auditory examination was performed in the schools by a specially trained nurse using an audiometer (Tegnér PTA 9) with double earphones The testing was made at 250 500 1000 2000 4000 an

Table 5 *Auditory examination*

Hearing impairments	Not examined at 4 years $n=439$ (category not 4)		Examined at 4 years $n=210$ (category 4)		Functionally important hearing impairments at 4 years	Sum $n=649$	
	n	%	n	%	n	n	%
(*a*) Referred	20	4.5	8	3.8	0	26	4.0
(*b*) Normal findings when examined by the audiologist	10	2.3	1	0.5	0	11	1.7
(*c*) Mild middle ear infections	4	0.9	2	0.9	0	6	0.9
(*d*) Severe middle ear infections	1	0.2	2	0.9	0	3	0.5
(*e*) Cerumen	2	0.5	0	0	0	2	0.3
(*f*) Slight sensorineural hypoacusis	1	0.2	2	0.9	0	3	0.5
(*g*) Moderate sensorineural hypoacusis	1	0.2	0	0	0	1	0.2
(*h*) Severe sensorineural hypoacusis	1	0.2	1	0.5	1	2	0.3
Sum of functionally important hearing impairments (*d g h*)	3	0.7	3	1.4	1	6	1.0
Newly detected	1	0.2	2	0.9	0	3	0.5
Previously known	2	0.5	1	0.5	1	3	0.5

category not 4 (1.9%) and in no one in category 4

Bacteriuria was revealed in 0.9% of the girls 1.4% in category not 4 and not at all in category 4 The difference in frequency between the two groups is not statistically significant ($p>0.05$) None of the children had bacteriuria at 4 years of age either No boy had bacteriuria

Altogether 42 seven year-old children entering school (6.5%) were considered to have functionally important physical health problems 27 (6.1%) in category not 4 and 15 (7.1%) in category 4 The difference between the two categories is not statistically significant ($p>0.05$)

Hearing

The frequency and kind of hearing impairments are shown in Table 5 Of the referred children 4.5% in category not 4 and 3.8% in category 4 ($p>0.05$) almost half had normal hearing when examined by the audiologist and about 1/3 had otosalpingitis Sensorineural hearing impairments were rare and the important ones had already been taken care of All children found to have otosalpingitis at 7 years of age were healthy at 4 years

Two children with slight sensorineural defects at 7 years had normal hearing at 4 years of age Three children with newly detected important impairment had all protracted middle ear infections

Vision

The distribution of visual disturbances is shown in Table 4 Significant eye disorders (groups 2 and 3) were found in 5.7% in category not 4 and in 11.4% in category 4 The difference is significant ($p<0.05$) Also the existence of previously known significant eye disorders was more common in category 4 ($p<0.001$) Newly discovered significant eye disorders were found in 3.9% and 2.4% respectively ($p>0.05$) (Table 6)

A comparison with the health control at 4 years of age shows that 24 of the school en

Table 3 *Weight for height in 210 children at 4 and 7 years of age*

Weight for height at 4 years	Weight for height at 7 years M+3 S D	M+2 S D	<M+2 S D	Sum
M+3 S D	(*a*) 3	(*b*) 1	(*c*) 0	(*d*) 4
M+2 S D	(*e*) 2	(*f*) 3	(*g*) 4	(*h*) 9
<M+2 S D	(*i*) 5	(*k*) 5	(*l*) 187	(*m*) 197
Sum	(*n*) 10	(*o*) 9	(*p*) 191	(*r*) 210

Limit +3 S D
Sensitivity $(a/n)\times 100=33\%$
Specificity $(f+g+k+l)/(o+p)\times 100=99\ 5\%$
Positive predictive value $(a/d)\times 100=75\%$

Limit +2 S D
Sensitivity $(a+b+e+f)/(n+o)\times 100=47\%$
Specificity $(l/p)\times 100=98\%$
Positive predictive value $(a+b+e+f)/(d+h)\times 100=69\%$

(3 9%), 12 girls and 13 boys the weight exceeded+3 S D (obesity) (Table 2) Ten of these 25 obese children were examined at 4 years of age Their mean weight at that time was 22 4 kg which is more than the mean weight for all 4 year olds (17 2 kg $p<0\ 001$) In three of them the weight for height exceeded+3 S D and was already at that time considered as a functionally important health problem Dietary treatment had been instituted and was continued sporadically but without great success

Out of the remaining children who had at 7 years a weight for height exceeding the standard with 2 S D 9 were examined at 4 years of age 3 were then at+2 S D and 1 above+1 S D In category not 4 8 children were reported to have been treated for obesity before school began also without success

Table 3 where the relation between weight and height at 7 years is compared with the same relation at 4 years of age can be used to compute the possibilities to predict at 4 years of age the risk of overweight at 7 years of age If +3 S D is used as the lower limit of obesity the sensitivity of the measurement will be 33% and the specificity 99 5% i e one third of the children who are obese at 7 years were obese already at 4 years and only one out of 200 7 year olds with normal weight was overweight at 4 years The predictive value of measurement is 75% i e 3 out of 4 children who were obese at 4 years will also be obese at 7 years If the limit of overweight is instead drawn at +2 S D the sensitivity will be 47% the specificity 98% and the predictive value 69%

Retentio testis was found in 3 of the boys in

Table 4 *Vision examination*

Eye disorders	Not examined at 4 years n=439 (category not 4) n	%	Examined at 4 years n=210 (category 4) n	%	Functionally important eye disorders at 4 years n	Sum n=649 n	%
Group 0	13	3 0	3	1 4	0	16	2 5
Group 1	14	3 2	9	4 3	1	23	3 5
Group 2	12	2 7	10	4 8	8	22	3 4
Group 3	13	3 0	14	6 7	11	27	4 1
Sum functionally important disorders (groups 2 and 3)	25	5 7	24	11 4	19	49	7 5
Newly detected	17	3 9	5	2 4		22	3 4
Previously known	8	1 8	19	9 0	19	27	4 1

tation inside and outside school but also what possibilities for care and alleviation can be offered by the society This means that in our country with its excellent economic social and medical resources heavy demands on optimal health could be made and thus that many deviations could be regarded as important to detect and treat

The deviations counted as important are listed in detail in Tables 1 and 4 and described in the classifications under *methods* The diagnoses and the classifications have been adapted as closely as possible to these used in the health control of 4 year old children (11 12 13 14)

The general health of the children was very good and severely handicapping disorders were detected and cared for already before school Slight motor disturbances with clumsiness and unskillfulness in performing the motor tests were rather common but were mostly regarded as normal variations Only in exceptional cases were they considered as important for the child s health and adaptation None of the 13 children with such a slight motor deviation had functionally important motor disturbances at 4 years of age In 3 children this clumsiness was combined with excessive mobility restlessness short attention span and hyperactivity i e what is usually called minimal brain dysfunction MBD

A closer penetration of these MBD-children and their behaviour at home and in school is necessary to be able to evaluate their special need for medical and educational support and help A follow up of a larger material of MBD-children is in progress

The frequency of *retentio testis* (3/223) in category not 4 does not differ significantly from that found in the health control of 4 year-olds (12/1972) (13) In category 4 no boy with testicular retention was found and with regard to the risk of infertility and malignancy it would have been more favourable for the 3 boys in category not 4 if their retentio testis had been detected and treated already in preschool age (17)

Bacteriuria was as frequent as in other studies of this age group—about 1% (10 14 23 24) Also as usual no boys with bacteriuria were discovered

Obesity in children is usually recognised as a precursor of obesity in adults which has evident negative effects on morbidity and mortality (18) An early identification of children at risk may therefore be important Several studies (1 5 6) indicate that rapid weight gain in infancy may be connected with obesity later in childhood However recent data from Swedish children do not confirm these findings (19) The actual frequencies of overweight defined as 2 S D above mean weight for height (9 1%) and obesity defined as 3 S D above mean weight for height (3 9%) are somewhat higher than in other studies of the same age groups of children (9 22) The increasing frequency of overweight from preschool age to school age is well known (18 20) as is also the fact that there is a tendency for obesity to persist from infancy throughout the preschool and school years (1 6 18)

However the possibility to predict with accuracy in preschool age who will be obese in school seems to be limited Thus only 1/3 of the obese school entrants were obese at 4 years of age and hardly any of the overweight ones were overweight at 4 years Besides still almost 20% of the overweight 4 year old children had normal weight at 7 years

The time for follow up is of course too short and the number of children in the study too small to allow definite conclusions to be drawn about riskgroups of obesity in childhood However it is evident that overweight is frequent in school children and that the number of overweight children increases during the later part of the preschool years Apparently measures for treatment and prevention have not been sufficient

In total 15 0% of the school entrants had functionally important health problems which is well in accordance with the findings in the preschool age (15 0%) and with the statement that the prevalence of chronic conditions in

Table 6 *Functionally important health problems in school entrants*

	Not examined at 4 years $n=439$ (category not 4)		Examined at 4 years $n=210$ (category 4)		Statistical difference	Sum $n=649$	
	n	%	n	%		n	%
Physical examination	27	6 1	15	7 1	$p>0.05^{NS}$	42	6 5
Newly detected	15	3 4	7	3 3	$p>0.05^{NS}$	22	3 4
Previously known	12	2 7	8	3 8	$p>0.05^{NS}$	20	3 1
Vision examination	25	5 7	24	11 4	$p<0.05^{*}$	49	7 5
Newly detected	17	3 9	5	2 4	$p>0.05^{NS}$	22	3 4
Previously known	8	1 8	19	9 0	$p<0.001^{***}$	27	4 1
Auditory examination	3	0 7	3	1 4	$p>0.05^{NS}$	6	1 0
Newly detected	1	0 2	2	0 9	$p>0.05^{NS}$	3	0 5
Previously known	2	0 5	1	0.5	$p>0.05^{NS}$	3	0 5
Total	55	12 5	42	20 0	$p<0.05^{*}$	97	15 0
Newly detected	33	7 5	14	6 7	$p>0.05^{NS}$	47	7 3
Previously known	22	5 0	28	13 3	$p<0.001^{*}$	50	7 7

trants with significant eye disorders had been examined earlier and that 3 of them had a normal visual screening test at that time All these 3 children were now at 7 years amblyopic in one eye one boy had a pronounced corneal astigmatism one had hyperopia and an excentric fixation and one had an eye muscle paresis It is very likely that these deviations did exist already at 4 years of age although they were not detected at the screening

Another 2 children had slight refractive errors at 4 years (astigmatism) and had not been considered in need of treatment at that time (group 1) In school however the demand for visual acuity is greater and they received glasses (group 2) Further on 3 children with significant eye disorders at 4 years (hyperopia astigmatism) had normal visual acuity at 7 years evidently a result of treatment An analysis of treated eye disorders in a greater number of school children is under preparation

In summary 15% of the 649 children entering school were found to have important health problems by either of the three methods of investigation—physical examination vision screening and hearing screening (Table 6) Visual defects were responsible for the largest part 7 5% while hearing defects were most infrequent 1% About half of the important health problems (51 5%) were previously known

Of the 210 children examined both at 4 and 7 years 42 (20%) had functionally important health problems at 7 years (Table 6) Two thirds of these defects were discovered already at the health examination at 4 years of age and were being treated and controlled The 14 newly detected children include 7 with obesity 2 with middle ear infection and 5 with visual defects Two of the children with visual defects were previously diagnosed as belonging to group 1 i e treatment was probably not necessary until school began

A pervading characteristic of Table 6 is that children who have passed the health control at 4 years of age have fewer newly detected important health problems and more previously known ones than other children This difference is most marked regarding previously known eye disorders

DISCUSSION

It can always be questioned which deviations from health should be considered as important for the child In this consideration it must be taken into account not only what influence the deviation may have on the child and his adap

tation inside and outside school but also what possibilities for care and alleviation can be offered by the society This means that in our country with its excellent economic social and medical resources heavy demands on optimal health could be made and thus that many deviations could be regarded as important to detect and treat

The deviations counted as important are listed in detail in Tables 1 and 4 and described in the classifications under *methods* The diagnoses and the classifications have been adapted as closely as possible to these used in the health control of 4 year old children (11 12 13 14)

The general health of the children was very good and severely handicapping disorders were detected and cared for already before school Slight motor disturbances with clumsiness and unskillfulness in performing the motor tests were rather common but were mostly regarded as normal variations Only in exceptional cases were they considered as important for the child s health and adaptation None of the 13 children with such a slight motor deviation had functionally important motor disturbances at 4 years of age In 3 children this clumsiness was combined with excessive mobility restlessness short attention span and hyperactivity i e what is usually called minimal brain dysfunction MBD

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most populations under 20 lies between 10% and 15% (14, 21) Visual defects dominate and hearing impairments are most uncommon as was also the case among children 4 years of age

The aim of the Child Health Services is to provide all children from birth to school age with a complete health surveillance To in crease the possibilities to fulfil this aim a gen eral health control of all 4 year old children was introduced in Sweden in 1969 (7 14) promoted by the handicap organisations Re sults from this health control in different parts of the country have shown unanimously that the health of preschool children is very good and that the majority of the seriously handi capping disorders are detected and treated already before 4 years of age although certain areas of the health surveillance are neglected *e g* dental health, vision hearing behaviour and upbringing (9, 14 15 24, 25 26)

With this background it is not probable that a follow up examination at 7 years of age would reveal large unknown defects of children s health Nevertheless, it would be of great interest to repeat the examination after 3–4 years and to let it serve as an evaluation of the efficiency of earlier examination It would be possible to find out whether a newly de tected health problem really was newly de veloped or had existed before but not been discovered The tendency to find fewer newly detected important health problems and more previously known ones in children who had been examined at 4 years of age compared with those who were not is an indication of the diagnostic capacity of the health control of 4 year olds Children with visual defects motor disturbances bacteriuria and testis re tention were detected and treated earlier than otherwise would have been the case On the other hand, the difference between the two categories was not very great and it is evident that also the ordinary health surveillance of preschool children has fulfilled its purpose in an acceptable way The greatest difference be tween the two categories was found among the visual defects naturally enough since visual defects in this age seldom are detected unless the visual acuity is examined In fact, 2 of the 5 children with newly detected visual defects had already been diagnosed at 4 years as de fects perhaps necessary to treat in school but not before

Nevertheless 3 children with amblyopia in one eye were missed when screened at 4 years The exact cause of these failures are not known—it might be a defective covering of the non tested eye—but it is evident that a certain under referral (as well as a certain over refer ral) is inevitable in screening procedures However the results have led to a check on the screening methods

The other previously examined children with newly detected functionally important health problems, had deviations that un doubtedly developed between 4 and 7 years of age (obesity and middle ear infections) Thus Child Health Services have fulfilled their func tion to detect handicapping disorders in pre school children By the introduction of the special health control at 4 years of age this function is further improved However also after the age of 4 years deviations occur that are important to detect and treat early and this means that the health surveillance should continue and be repeated also during the later part of the preschool period

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Submitted May 28 1976
Accepted Oct 1? 1976

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Acta Pædiatr Scand 66 307–310 1977

PHYSICAL MASS EXAMINATIONS IN THE SCHOOL HEALTH SERVICE

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ABSTRACT Köhler L. (Department of Paediatrics University Hospital Lund and Dalby Community Health Research Centre Dalby Sweden) Physical mass examination in the school health service Acta Paediatr Scand 66 307 1977.—At 7 years of age all 649 children in a school district underwent a physical examination a vision screening and an auditory screening The purpose of the present study was to analyse the value of the routine physical examination within the school health services In 15% of the children functionally important health problems were found Visual defects were most common comprising 7 5% then came physical health problems such as motor disturbances obesity bacteriuria in 6 5% and hearing defects in 1% About half of the important health problems were previously known Most disorders of importance were detected by the nurse s screening examination and rather few by the doctor s physical examination It seems advisable to introduce screening procedures in the hands of nurses also for the physical examination The role of the school physician in the general health surveillance would then be mainly to control and verify specific observations or suspicions of disease or handicap noted by the school nurse His time and attention could instead be directed towards important tasks which are now often neglected e g health education and care of sick and handicapped children in the school setting

KEY WORDS School health screening examinations

Health examination of school-children is one of the few compulsory health examinations in Sweden According to regulations routine physical examinations should be performed on all children in certain classes usually 1 4 7 and 9 i e at 7 10 13 and 15 years of age Besides special consultations should be arranged by the school physician and/or the school nurse for children in need of such contacts

Although other aspects of the children s health and welfare have been added a great part of the doctor s time is still taken up by routine physical examinations However the value of such periodic medical inspections is not properly documented Studies mostly from England and USA (1 7 8 9 10 11) give an almost depressing picture of the efficiency and usefulness of such examinations In Sweden practically no efforts have been made to evaluate what is achieved in the school health services A recent small study from Uppsala concludes that the results of the clinical examination of children when they enter school are very sparse and a restriction of this item is recommended (2)

The purpose of the present paper is to discuss the aims and methods of the school health service as they were elucidated in an epidemiological study of the physical health of 7 year old entrants (5)

MATERIAL AND METHODS

In the first class at the age of 7 years 649 children born 1967–1965 and living in the school district of Dalby in Southern Sweden were examined

screening examinations of vision hearing urine weight and height (43 children or 6 7%) Also the physician discovered some new im portant deviations but they were rather few (4 children or 0 6% 3 boys with retentio testis and 1 with minimal brain dysfunction)

DISCUSSION

Medical care of children in the school setting is provided in routine class examinations in consultations and in case conferences and is mainly directed towards prevention Consulta tions and conferences are meant for children with special health problems and also for children at risk while the early discovery of disease and handicap is concentrated mainly on the regular routine mass examinations usually at 7 10 13 and 15 years of age These examinations include questionnaires about previous and actual diseases measuring of weight and height and a somatic check up by the physician Besides the school nurse screens for abnormalities of vision hearing and urine

The present study may be regarded as one of these routine health examinations Since the study comprised the total number of 7 year-old children in the area and since all children were examined by the same staff who used standardised methods and uniform classifications it is possible to make a reliable evaluation of the results

It is evident that the yield from the physi cian s examinations was not very high and it is also clear that the methods used by the physi cian were time consuming and expensive (clinical examination)

Of course there are other aspects of routine check ups that may make them valuable for doctors parents and children e g the op portunities of establishing a positive first con tact for the benefit of future cooperation

These more subtle considerations of health care are also important and they could be pro vided for e g by conferences meetings les sons and demonstrations However it does not seem appropriate to devote that much time and money to medical performances with so low a yield and to detect among healthy children deviations in need of medical evalua tion and treatment there are other and better methods than the traditional clinical examina tion

Screening methods can be used more exten sively also here e g by letting the nurse make a short physical appraisal according to standardised questionnaires and observation forms (1 3 6) Based on these observations the school physician performs an assessment on selected children and the clinical examina tion then serves as a purposeful diagnostic method Thus the school physician will sel dom or never use clinical examinations as screening methods to identify health prob lems but he will use them for assessment based on specific observations or suspicions of disease or handicap A reduction of the number of physical mass examinations will mean that more of the school physician s time and attention can be paid to important tasks which are now often neglected e g to health education of both teachers and pupils and to vocational guidance It could also mean that more time could be found for increased medi cal and co-ordinating responsibility within the school setting for children with chronic dis eases and handicaps and perhaps also for better possibilities to treat sick children

In this way school health services would provide health care that is more tailored to the needs of the individual than it is today

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Table 1. *Classification of health problems*

Method of examination	Group 0 Healthy child	Group 1 Slight deviation without importance	Group 2 Moderate deviation. Treatment indicated	Group 3 Definitely handicapping disorder
General physical examination		*Flat feet*, phimosis	Retentio testis, MBD, obesity	Cerebral palsy, organic heart disease
Vision examination		*Myopia* <−1.0 D Hyperopia <+1.5 D *Astigmatism* <±1.5 D Slight heterophoria	Refractive errors without amblyopia. Heterophoria with complaints	Amblyopia, strabismus
Auditory examination		Mild otosalpingitis cured by simple otological measures and few visits to the physician	Protracted otosalpingitis requiring more intense therapy (adenoidectomy, drainage tubes). Moderate sensorineural hearing impairment with need for follow-up and control	Severe impairment requiring hearing aid or special education

According to a structured and standardised form the children's physical health was examined by the author. Screening of vision, hearing, urine and measurements of height and weight were performed by the school nurse. A more detailed description of the methods has been given previously (5). Children with health problems considered to be functionally important, at present or in the future and not under current professional care, were referred for further evaluation via the ordinary school physician. After evaluations and diagnosis, the health problems were graded according to their importance for the children's health and development (Table 1) (4).

Groups 2 and 3 were considered as *functionally important health problems* (5). *Previously known* means that the health problems had already been detected and cared for before the actual investigation.

Table 2. *Physical examination*

Health problems	n=649 n	 %
(*a*) Motor disturbance group 1	23	3.5
(*b*) Motor disturbance group 2	7	1.1
(*c*) Motor disturbance group 3	1	0.2
(*d*) Obesity >+3 S.D.	25	3.9
(*e*) Bacteriuria	3	0.9
(*f*) Multiple malformations	2	0.3
(*g*) Retentio testis	3	0.9
(*h*) Phimosis	2	0.3
(*i*) Inguinal hernia	2	0.3
(*k*) Adenoid	1	0.2
(*l*) Organic heart disease	1	0.2
(*m*) Flat feet		
Sum of functionally important health problems (all in *b*, *c*, *d*, *e*, *f*, *g*, *l*)	42	6.5
Newly detected (in *b*, *d*, *e*, *g*)	22	3.4

RESULTS

Out of the 649 children entering school 15% were found to have important health problems by either of the three methods of investigation—physical examination, vision screening and hearing screening (Tables 2 and 3). Visual defects were responsible for the largest part, 7.5%, while hearing defects were most infrequent, 1%. About half of the important health problems (51.5%) were previously known. Details of the findings are given in a previous paper (5).

Most deviations of importance for the children's health were detected by the nurse's

Table 3. *Functionally important health problems in school entrants*

	n	%
Physical examination	42	6.5
Newly detected	22	3.4
Previously known	20	3.1
Vision examination	49	7.5
Newly detected	22	3.4
Previously known	27	4.1
Auditory examination	6	1.0
Newly detected	3	0.5
Previously known	3	0.5
Total	97	15.0
Newly detected	47	7.3
Previously known	50	7.7

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DISCUSSION

Medical care of children in the school setting is provided in routine class examinations in consultations and in case conferences and is mainly directed towards prevention Consultations and conferences are meant for children with special health problems and also for children at risk while the early discovery of disease and handicap is concentrated mainly on the regular routine mass examinations usually at 7 10 13 and 15 years of age These examinations include questionnaires about previous and actual diseases measuring of weight and height and a somatic check up by the physician Besides the school nurse screens for abnormalities of vision hearing and urine

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Previously known	3	0.5
Total	97	15.0
Newly detected	47	7.3
Previously known	50	7.7

Acta Pædiatr Scand 66 311–315 1977

EXCESSIVE HEPATIC GLYCOGEN STORAGE IN GLUCOSEPHOSPHATE ISOMERASE DEFICIENCY

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ABSTRACT Van Biervliet J P G M and Staal G E J (University Children s Hospital Het Wilhelmina Kinderziekenhuis Utrecht The Netherlands) Excessive hepatic glycogen storage in glucosephosphate isomerase deficiency Acta Paediatr Scand 66 311 1977.—Excessive amounts of glycogen were found in liver and erythrocytes of a patient suffering from generalized glucosephosphate isomerase deficiency A low carbohydrate diet fre quent meals and avoidance of peak carbohydrate challenges resulted in a significant decrease of liver volume without affecting the haematological condition The possible mechanism of these findings are discussed

KEY WORDS Glucosephosphate isomerase deficiency glycogen storage

Glucosephosphate isomerase (GPI) (E C 5 3 1 9) catalyses the reversible interconver sion of glucose-6-phosphate (G 6 P) and fructose 6-phosphate (F 6-P) Human GPI has been shown to exist in several rare electro-phoretic forms and in at least 18 physico chemically different deficient variants (17) So far very little attention has been given to the effects of GPI deficiency on organ systems other than blood cells Evidence of excessive glycogen storage in liver tissue and erythro cytes was obtained Differentiation from other liver glycogen storage diseases is discussed Until now no reports on increased liver glycogen have been published

CASE REPORT

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METHODS

Carbohydrate metabolism was studied in vivo by different oral and parenteral tolerance tests as described by Fernandes et al (? 3 4) for differentiation of liver glycogenosis Glycogen was determined in erythrocytes after isolation by heating in alkali degradation by α-glucosidase amylase (6) and analysis of glucose by the hexokinase reaction Liver biopsy specimens were pro cessed for ultrastructural studies by current procedures Methods used for enzyme activity determinations and for study of the GPI kinetics and enzyme properties have been described elsewhere (5 14 15)

RESULTS

Morphological studies

A liver biopsy was performed after a fasting episode of 15 hours normally sufficient for exhaustion of hepatic glycogen stores In the proband however abundant cytoplasmic glycogen stored as beta particles was still pres

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Submitted May 28 1976
Accepted Oct 12 1976

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Acta Pædiatr Scand 66 311–315 1977

EXCESSIVE HEPATIC GLYCOGEN STORAGE IN GLUCOSEPHOSPHATE ISOMERASE DEFICIENCY

J P G M VAN BIERVLIET and G E J STAAL

From the University Children s Hospital Het Wilhelmina Kinderziekenhuis and the Unit of Medical Enzymology Academic Hospital of the Dutch State University Utrecht The Netherlands

ABSTRACT Van Biervliet J P G M and Staal G E J (University Children s Hospital Het Wilhelmina Kinderziekenhuis Utrecht The Netherlands) Excessive hepatic glycogen storage in glucosephosphate isomerase deficiency Acta Paediatr Scand 66 311 1977.— Excessive amounts of glycogen were found in liver and erythrocytes of a patient suffering from generalized glucosephosphate isomerase deficiency A low carbohydrate diet frequent meals and avoidance of peak carbohydrate challenges resulted in a significant decrease of liver volume without affecting the haematological condition The possible mechanism of these findings are discussed

KEY WORDS Glucosephosphate isomerase deficiency glycogen storage

Glucosephosphate isomerase (GPI) (E C 5 3 1 9) catalyses the reversible interconversion of glucose-6 phosphate (G-6-P) and fructose-6-phosphate (F-6-P) Human GPI has been shown to exist in several rare electrophoretic forms and in at least 18 physicochemically different deficient variants (17) So far very little attention has been given to the effects of GPI deficiency on organ systems other than blood cells Evidence of excessive glycogen storage in liver tissue and erythrocytes was obtained Differentiation from other liver glycogen storage diseases is discussed Until now no reports on increased liver glycogen have been published

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Carbohydrate metabolism was studied in vivo by different oral and parenteral tolerance tests as described by Fernandes et al (2 3 4) for differentiation of liver glycogenosis Glycogen was determined in erythrocytes after isolation by heating in alkali degradation by α glucosidase amylase (6) and analysis of glucose by the hexokinase reaction Liver biopsy specimens were processed for ultrastructural studies by current procedures Methods used for enzyme activity determinations and for study of the GPI kinetics and enzyme properties have been described elsewhere (5 14 15)

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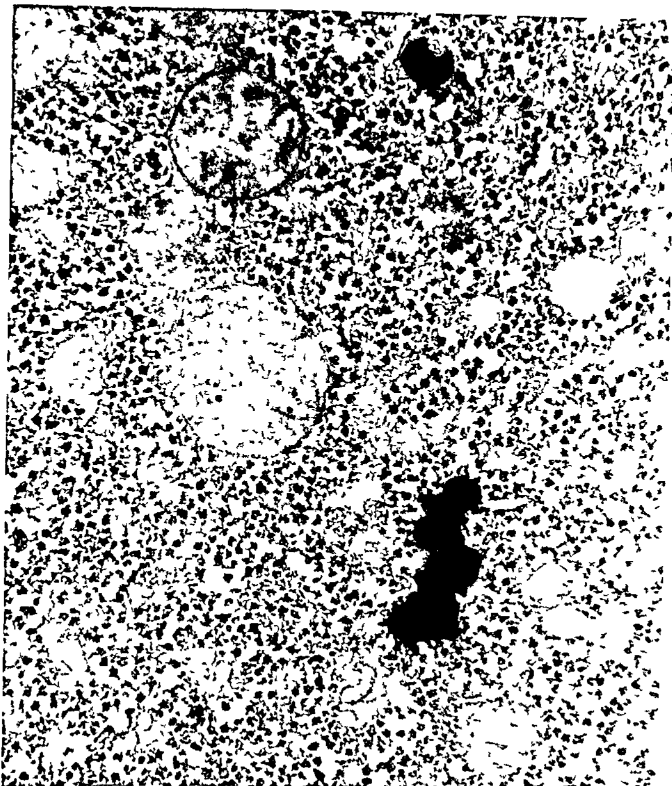


Fig 1 Electronmicroscopy of hepatic tissue The biopsy was obtained after a fasting of 15 hours Excessive amounts of glycogen stored as β particles is obvious Besides sporadic increase of mitochondrial diameter no other abnormalities could be observed No excessive fat deposition was seen (×20 000)

ent (Fig 1) The mean mitochondrial volume was normal Some mitochondria however were enlarged No excessive fat deposition was observed

Erythrocyte glycogen content

A significant increase in erythrocyte glycogen content was seen 190 µg/g Hb controls 23–64 µg/g Hb The mean corpuscular haemoglobin

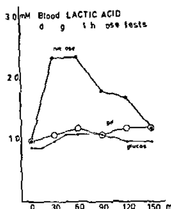


Fig 2 Comparison of the lactate response to different oral hexose tolerance tests Note the important difference of the lactate response between galactose metabolised to G 1 P glucose metabolised to G-6-P both located before the deficient GPI step and the fructose metabolised to products behind this step These tests suggest the diversion of glucose and galactose towards the glycogen pools

concentration was normal 2 050 fmole (controls 1 720–2 170)

Dietary results

A low carbohydrate diet frequent meals and avoidance of peak carbohydrate challenges resulted in a marked decrease in liver volume After 6 months of this regimen the liver volume was entirely normalized These measures however did not influence the course of the haematological condition

Studies of the carbohydrate metabolism

After an oral glucose test normal responses for glucose insulin and free fatty acids (FFA) but low levels of lactic acid (LA) were observed (Fig 2) Intramuscular *glucagon* administration was followed by a quite normal glycogenolytic response

The galactose tolerance test showed low LA response normal galactose clearing no glucose response and a very small FFA response indicating rapid disappearance of galactose towards the glycogen pools and absence of the usual stimulation of glycolysis normally resulting in a small but significant increase of LA levels (Fig 2)

The fructose tolerance test revealed a marked increase of LA and no glucose response indicating diversion of fructose towards the glycolytic sequence below the deficient GPI step (Fig 2)

These results exclude the presence of liver glycogenosis caused by deficiencies of glucose-6-phosphatase or branching and debranching enzymes and of the enzymes of the phosphorylase system (2 3 4) The normal LA increase after standardized muscular exercise compared to controls of the same age excludes muscle phosphorylase deficiency

Considering however the untrained muscular condition of our proband LA formation should have been much higher in the absence of a glycolytic disorder (8) Extensive studies on erythrocytes and leucocytes showed low GPI activity but normal or increased values of all other glycolytic enzymes (e g phosphofructokinase (PFK) this excluding PFK deficiency as a cause of glycogen storage (13)

DISCUSSION

The glycogen storage in this patient is caused by unbalance between carbohydrate intake and the metabolic capacity of the *glycolytic* sequences Normally the reversible reactions of glycolysis rarely become rate limiting for either glycolysis or gluconeogenesis (11) Considering the high molecular activity and the amounts of GPI normally large enough to maintain the reaction G-6-P↔F 6-P near its *equilibrium* one could wonder why 26% residual activity results in important cellular *dysfunction* In our patient however this equilibrium is not achieved This was demonstrated in erythrocytes by the increased [G-6-P]/[F 6 P] quotient (5 1 controls 3 1±0 5) (17) in the presence of 24% residual activity During a glycolytic flux G-6 P accumulates in hepatic GPI deficiency and four groups of enzymes compete for this substrate Embden Meyerhof hexose monophosphate (HMP) shunt glycogenic and gluconeogenic enzymes A plausible explanation of the in

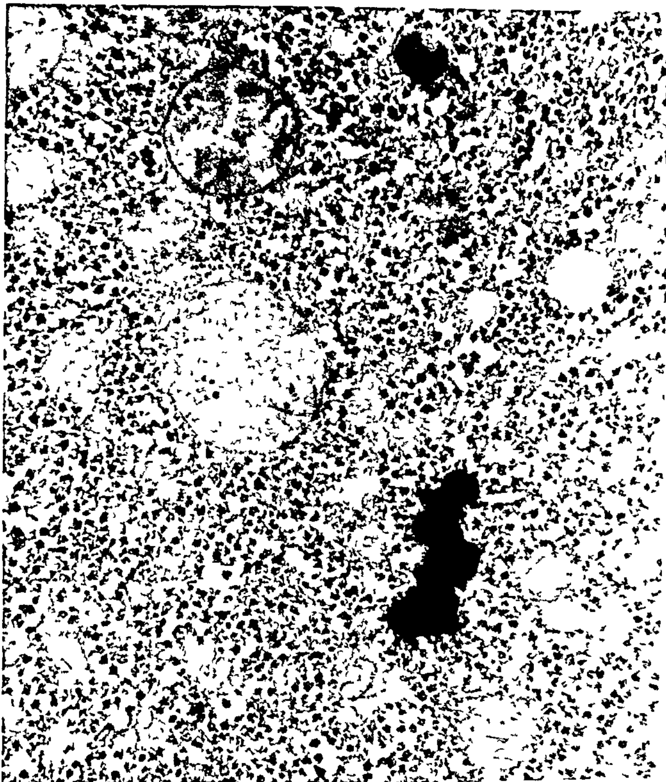


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Submitted May 18 1976
Accepted Oct 1 1976

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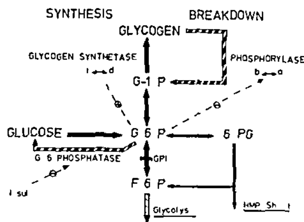


Fig 3 Mechanism of glycogen storage in glucose phosphate isomerase deficiency
G 1 P=glucose 1 phosphate G-6-P=glucose-6 phosphate F-6-P=fructose-6-phosphate 6-PG=6 phosphogluconate HMP=hexosemonophosphate shunt GPI=glucosephosphate isomerase

creased glycogen deposition could be found considering the limited metabolic capacity of the pathways normally metabolizing G 6 P The strong competitive inhibition of GPI by acyclic sugar phosphates possessing a free carbonyl group provides a negative feed back mechanism for the control of the Embden Meyerhof pathway (1) and further limits its capacity in GPI deficiency Also the diversion of G-6 P through the HMP shunt is limited by the competitive inhibition of NADPH on glucose 6 phosphate dehydrogenase (G 6 PD)

In hepatocytes the synthesis of G 6 PD could be stimulated by insulin (18 21) In contrast with our patients with glucose 6 phosphatase deficiency the plasma insulin levels during an oral glucose tolerance test were not decreased Thus increased activity of the HMP shunt could result from increased synthesis of G 6-PD However this also increases the amounts of 6 phosphogluconate erythrose-4 phosphate and sedoheptulose 7 phosphate intermediates of the HMP shunt and strong inhibitors of the GPI reaction (1) G 6-P can be dephosphorylated by glucose 6 phosphatase but the normal insulin activity results in normal depression of gluconeogenesis and promotion of glycogen synthesis and glycolysis by increasing or depressing the biosynthesis of their key enzymes (18 19 20, 21) In this way more substrate is presented to the glycogenic pathways Increased levels of G 6 P promote the interconversion of glycogen synthase to its active form and partially inhibit some steps in the phosphorylase system, this further promotes glycogen deposition (6 12) (Fig 3) The stored glycogen however can be mobilized as demonstrated during a glucagon test The disturbed equilibrium between carbohydrate intake and its disposal can be restored by restriction of carbohydrate and avoidance of peak challenges Such treatment resulted in normalization of liver volume after 6 months Besides the disorders of the enzymes of glycogen metabolism (phosphorylase phosphorylase kinase branching—& debranching enzyme and α glucosidase) glycogen storage can also occur as a secondary phenomenon in glucose 6 phosphatase and muscle PFK deficiency GPI deficiency can be classified in this cathegory

The absence of tissue specific GPI isoenzymes facilitates differentiation from other glycogen storage diseases A non spherocytic haemolytic disorder was observed in all GPI deficiencies so far described Most liver glycogen storage diseases result in liver enlargement Only moderate enlargement was present in our proband

In our patient muscular fatigue a complaint in muscle phosphorylase and muscle PFK deficiency was also present Apart from our patient liver GPI activity has never been measured in GPI deficiency Matsumoto et al (9) however noted the presence of abundant glycogen granules in the hepatic parenchymal cells of a boy with GPI Narita Mrozek et al (10) described a glycogen myopathy in a 47 year old patient suffering from muscular fatigue Defective activity of GPI was observed but PFK activity was not measured The absence of haemolytic disease could suggest an M type PFK deficiency and inhibition of GPI by a mechanism similar to that observed in Tarui s pa

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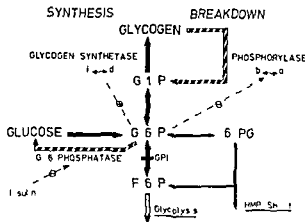


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Acta Pædiatr Scand 66 317–322 1977

SISOMICIN TREATMENT OF SERIOUS NEONATAL INFECTIONS

A Clinical and Pharmacokinetic Study

P HENRIKSSON N SVENNINGSEN G KAHLMETER and K HAEGER

From the Departments of Paediatrics Malmo General Hospital Malmo and the University Hospital Lund and the Institute of Microbiology University of Lund Lund Sweden

ABSTRACT Henriksson P Svenningsen N W and Haeger K (Departments of Paediatrics University Hospital Lund and Allmanna Sjukhuset Malmo Sweden) Sisomicin treatment of serious neonatal infections A clinical and pharmacokinetic study Acta Paediatr Scand 66 317 1977 —23 infants 20 of which had verified or clinically highly suspected serious infections in the neonatal period were treated with a new antibiotic aminoglycoside sisomicin i m in doses from 2 8 to 6 6 mg/kg/24 h Clinical cure was obtained in 18 of 20 cases and marked impro ement in one case Adverse effects were only observed in two infants with tenderness at the injection sites Serum concentrations and half-life estimations showed that the concentrations were similar to those obtained for gentamicin and that half-life was independent of postnatal age but highly correlated to the gestational age and to body weight Consideration should therefore be given to the prolonged half-life of the drug in immature and low birth weight infants

KEY WORDS Aminoglycosides antibiotic treatment infections neonatal period pharmacokinetics sisomicin

Sisomicin is an antibiotic produced at the growth of *Micromonospora inyoensis* It is an aminoglycoside nearest related to the C_{1a} fraction of gentamicin the properties of which it shares to a certain extent However *in vitro* experiments and animal experimentation pointed towards a greater activity of sisomicin (4 16) Laboratory findings (16) hinted that sisomicin in therapeutic doses might have a lower ototoxic and nephrotoxic effect than that of other aminoglycosides

Only 2 clinical studies on sisomicin in the paediatric age group have been reported (3 14) The purpose of the present study was to evaluate the efficacy and tolerance of sisomicin treatment of infants with serious neonatal infections as well as to estimate the serum half life (*T/2*) in relation to gestational and postnatal age

MATERIAL AND METHODS

Twenty three newborn infants with serious systemic infections were studied The distribution of age and sex gestational week body weight diagnosis and causative organism is given in Table 1 In most cases the infection occurred concomitantly with another disease i e perinatal asphyxia (5 infants) idiopathic respiratory distress syndrome (IRDS) (10 infants) and one each with cardiac decompensation hemolytic disease (Rh immunization) recto-vaginal fistula hypoglycemia hyperbilirubinemia hypogammaglobulinemia muscular hypotonia congenital stridor facial paralysis and pharyngeal incoordination with swallowing disturbance Respiration had to be assisted in eight infants Cardiotonic drugs were given in two cases and diuretics in one case Seven infants had umbilical vein catheter during at least part of the treatment period In one case the infant received exchange blood transfusions

Administration of antibiotic

At the beginning of this investigation little was known of the adequate dosage of sisomicin in neonates Individual dosage is presented in Table 1 In three cases

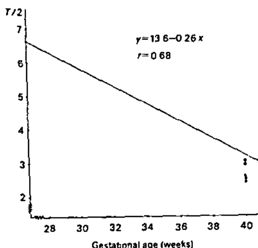


Fig 1 Serum half life (hours) in the first neonatal week in relation to maturity (gestational age) at birth

for gentamicin and tobramycin (7) Statistical calculations were made according to generally accepted principles (10)

RESULTS

Bacteriological results

The causative organism was identified in 15 cases (Table 1) and was found to be sensitive to sisomicin *in vitro* All these 15 patients reacted well on treatment with sisomicin only or a combination of sisomicin and ampicillin In 7 cases with positive culture sisomicin was the only anti infectious agent In one case of combined therapy the microorganisms were sensitive to sisomicin but not to ampicillin

Nine patients were treated with various antibiotics prior to sisomicin The results of these treatments are given in Table 2 Sisomicin treatment resulted in clinical cure in all these cases

Clinical results

In cases 2 4 7 9 and 14 the causative organism could not be isolated but hematological data (thrombocytopenia leucocytosis increased IgM or CRP) and pulmonary X ray verified the clinical signs of infection

In cases 6 8 11 infection was suspected at admission and antibiotic treatment was given but the diagnosis could not be verified neither on bacteriological nor hematological data The result of the treatment with sisomicin was thus classified as complete clinical cure in 18 of 20 cases (90%) In one case the treatment resulted in marked improvement This infant also suffered from bronchopulmonary dysplasia and hypogammaglobulinemia

In another case (no 18) all signs of clinical and bacteriological infection disappeared during treatment with sisomicin but the infant was re infected 11 days later Sisomicin was given again with bacteriologically and clinically excellent result

Adverse effects

In two patients there were signs of moderate tenderness at the injection site At follow up examinations at 6 to 14 months of age including neurological and developmental examination we did not observe any signs of auditory or vestibular damage In all patients hemoglobin white blood cells differential count platelets serum creatinine blood urea N protein and glucose in urine and microscopical urinalysis were routinely observed during and after treatment These laboratory data showed neither signs of nephrotoxicity nor hematological disturbances referable to sisomicin Fur

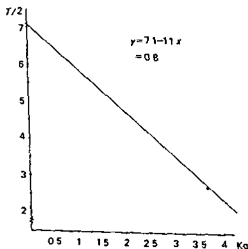


Fig 2 Serum half life (hours) Means per treatment period in individual patients vs body weight

Table 1 *Diagnosis and causative organism(s) in 23 newborns treated with sisomicin*

Pat no	Age (days)	Sex	Gestat week	Weight (g)	Diagnosis	Causative organism(s)	mg/kg/24 h	No of days treated	Additional antibiotic
1	1	m	40	3 100	Aspiration pneumonia	E coli	3.2	10	Ampicillin
2	1	m	40	2 900	Septicemia	Unknown	3.1	7	Ampicillin
3	1	f	29	1 100	Septicemia	E coli	3.6 5.4	3 16	Ampicillin
4	1	m	37	3 400	Pneumonia	Unknown	4.1	10	Ampicillin
5	2	m	40	3 100	Meningitis	E coli	4.8	8	–
6	2	m	31	1 570	Septicemia?	Unknown	2.8	10	Ampicillin
7	3	m	40	4 000	Pneumonia	Unknown	3.0	9	–
8	3	m	40	3 600	UTI? Septicemia?	Unknown	3.0	11	Ampicillin
9	4	f	38	3 100	Meningitis?	Unknown	3.2	11	Ampicillin
10	5	m	40	3 500	Septicemia UTI	E coli	5.1	10	Ampicillin
11	5	f	34	3 700	Septicemia?	Unknown	3.0	11	Ampicillin
12	5	f	37	2 300	Septicemia	Enterobacter Enterococci	3.0	11	Ampicillin
13	5	m	28	900	Pneumonia	E coli	6.6	16	–
14	6	m	36	2 200	Septicemia?	Unknown	3.6	6	Ampicillin
15	7	m	32	1 300	Septicemia	Klebsiella pneumoniae	3.0	17	–
16	7	m	39	3 460	Meningitis	E coli	4.3 4.3	6 9	Ampicillin
17	7	m	30	1 100	Septicemia	Enterobacter	5.4 3.6	4 13	–
18	7	m	40	2 800	Genitourinary infection Septicemia	Klebsiella Staph aureus	2.9 2.9 4.3	11 9 4	Ampicillin
19	7	m	40	3 800	Meningitis	Listeria monocytogenes	3.1	9	Ampicillin
20	8	f	30	1 320	Septicemia	E coli	4.6	10	Ampicillin
21	12	m	30	1 200	Septicemia	E coli	5.0 3.7	3 5	–
22	18	f	26	950	UTI Septicemia	Staph aureus Klebsiella	4.2	21	–
23	20	m	29	1 350	Septicemia	E coli	4.4	11	–

the daily dose was lower than 3 mg/kg In 13 treatment periods the daily dose was 3.0–3.9 mg/kg in 8 periods 4.0–4.9 mg/kg and in 5 periods greater than 5.0 mg/kg The total dose per child varied between 41 and 225 mg (mean 103±48.8 mg) All doses were administered as i m injection every 12 hours

In 15 cases ampicillin was given simultaneously with sisomicin The dose was usually 150 to 200 mg/kg/24 h in two or three injections Individual patients receiving ampicillin are listed in Table 1

Sisomicin concentrations in serum were determined with a modified agar well diffusion technique earlier used

Table 2 *Cases with previous ineffective antibiotic treatment*

No of cases	Species	Antibiotic	Results of prior treatment
1	Klebsiella	Ampicillin	Indeterminate
1	Klebsiella + Staph aureus	Cephalothin Gentamicin	Blood culture became normal urine culture still pos after 10 days on sisomicin normal
1	Pseudomonas	Gentamicin Ampicillin	Poor
1	E coli	Cephalothin	*Initially good but recurrent infection*
1	E coli	Cephalothin	Poor
2	E coli	Ampicillin	Indeterminate
1	E coli	Cephalexin	Poor
1	Enterobacter	Ampicillin	Poor

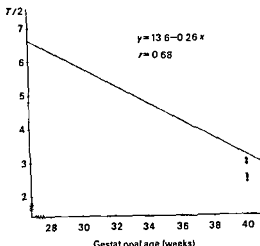


Fig 1 Serum half life (hours) in the first neonatal week in relation to maturity (gestational age) at birth

for gentamicin and tobramycin (7) Statistical calculations were made according to generally accepted principles (10)

RESULTS

Bacteriological results

The causative organism was identified in 15 cases (Table 1) and was found to be sensitive to sisomicin *in vitro* All these 15 patients reacted well on treatment with sisomicin only or a combination of sisomicin and ampicillin In 7 cases with positive culture sisomicin was the only anti infectious agent In one case of combined therapy the microorganisms were sensitive to sisomicin but not to ampicillin

Nine patients were treated with various antibiotics prior to sisomicin The results of these treatments are given in Table 2 Sisomicin treatment resulted in clinical cure in all these cases

Clinical results

In cases 2 4 7 9 and 14 the causative organism could not be isolated but hematological data (thrombocytopenia leucocytosis increased IgM or CRP) and pulmonary X ray verified the clinical signs of infection

In cases 6 8 11 infection was suspected at admission and antibiotic treatment was given but the diagnosis could not be verified neither on bacteriological nor hematological data The result of the treatment with sisomicin was thus classified as complete clinical cure in 18 of 20 cases (90%) In one case the treatment resulted in marked improvement This infant also suffered from bronchopulmonary dysplasia and hypogammaglobulinemia

In another case (no 18) all signs of clinical and bacteriological infection disappeared during treatment with sisomicin but the infant was re infected 11 days later Sisomicin was given again with bacteriologically and clinically excellent result

Adverse effects

In two patients there were signs of moderate tenderness at the injection site At follow up examinations at 6 to 14 months of age including neurological and developmental examination we did not observe any signs of auditory or vestibular damage In all patients hemoglobin white blood cells differential count platelets serum creatinine blood urea N protein and glucose in urine and microscopical urinalysis were routinely observed during and after treatment These laboratory data showed neither signs of nephrotoxicity nor hematological disturbances referable to sisomicin Fur

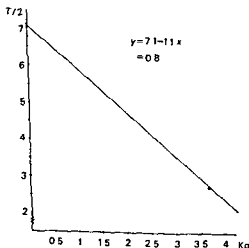


Fig 2 Serum half life (hours) Means per treatment period in individual patients vs body weight

Table 1 *Diagnosis and causative organism(s) in 23 newborns treated with sisomicin*

Pat no	Age (days)	Sex	Gestat week	Weight (g)	Diagnosis	Causative organism(s)	mg/kg/ 24 h	No of days treated	Additional antibiotic
1	1	m	40	3 100	Aspiration pneumonia	E coli	3 2	10	Ampicillin
2	1	m	40	2 900	Septicemia	Unknown	3 1	7	Ampicillin
3	1	f	29	1 100	Septicemia	E coli	3 6 5 4	3 16	Ampicillin
4	1	m	37	3 400	Pneumonia	Unknown	4 1	10	Ampicillin
5	2	m	40	3 100	Meningitis	E coli	4 8	8	–
6	2	m	31	1 570	Septicemia?	Unknown	2 8	10	Ampicillin
7	3	m	40	4 000	Pneumonia	Unknown	3 0	9	–
8	3	m	40	3 600	UTI? Septicemia?	Unknown	3 0	11	Ampicillin
9	4	f	38	3 100	Meningitis?	Unknown	3 2	11	Ampicillin
10	5	m	40	3 500	Septicemia UTI	E coli	5 1	10	Ampicillin
11	5	f	34	3 700	Septicemia?	Unknown	3 0	11	Ampicillin
12	5	f	37	2 300	Septicemia	Enterobacter Enterococci	3 0	11	Ampicillin
13	5	m	28	900	Pneumonia	E coli	6 6	16	–
14	6	m	36	2 200	Septicemia?	Unknown	3 6	6	Ampicillin
15	7	m	32	1 300	Septicemia	Klebsiella pneumoniae	3 0	17	–
16	7	m	39	3 460	Meningitis	E coli	4 3 4 3	6 9	Ampicillin
17	7	m	30	1 100	Septicemia	Enterobacter	5 4 3 6	4 13	–
18	7	m	40	2 800	Genitourinary infection Septicemia	Klebsiella Staph aureus	2 9 2 9 4 3	11 9 4	Ampicillin
19	7	m	40	3 800	Meningitis	Listeria monocytogenes	3 1	9	Ampicillin
20	8	f	30	1 320	Septicemia	E coli	4 6	10	Ampicillin
21	12	m	30	1 200	Septicemia	E coli	5 0 3 7	3 5	–
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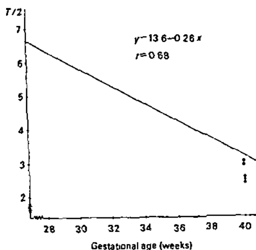


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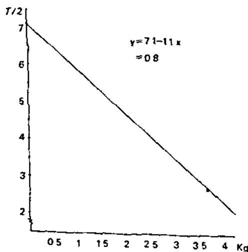


Fig 2 Serum half life (hours) Means per treatment period in individual patients vs body weight

Table 3 Average serum concentration of sisomicin in neonates

Single dose	P		P_{1h}		P_{2h}		P_{8h}	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D
≤1 75 mg/kg	1 21	0 94	4 42	1 49	3 23	1 48	1 57	0 76
≥1 75 mg/kg	1 44	0 35	4 03	1 66	3 03	1 37	1 92	0 97

thermore, in 7 infants glomerular filtration rate measured by single injection polyfructosan clearance test (17) within 4 weeks after sisomicin therapy was normal in all cases i e 30 5 to 59 0 ml/min/1 73 m² body surface

PHARMACOKINETICS

It is well known that serum half life of hitherto known aminoglycosides differs between children and adults (12, 15) Serum half life of sisomicin ($T/2$) was estimated by plotting the concentration of sisomicin in serum against time on semilogarithmic graph paper $T/2$ was calculated from

$$T/2=\frac{0\ 693}{\lambda}$$

in which λ is derived from

$$\log P_{siso}=\log P_{siso}0-\frac{\lambda}{2\ 303}\times t$$

For further details on half life calculation in aminoglycosides see Gyselynck et al (5)

Data for calculation of more than one half life estimation were obtained from 13 patients In these the average $T/2$ was calculated

There was no relation between the age in days and the $T/2$ according to the equation $y=4\ 3-1\ 5x$, $r=0\ 1$ However when $T/2$ was related to the gestational week at birth there was a significant correlation $T/2$ decreased by increasing maturity according to the equation $y=13\ 6-0\ 3x$, $r=0\ 68$ (Fig 1) There was also a correlation between body weight and $T/2$ Half life decreased by increasing body weight according to the equation $y=7\ 1-1\ 1x$ $r=0\ 8$ (Fig 2)

The average serum concentrations of sisomicin after a single dose are given in Table 3 Since clinical cure was complete in 90% of the cases it was not possible to correlate the clinical outcome to individual serum concentrations

DISCUSSION

It has been shown in this study that sisomicin in doses of 2 8–6 6 mg/kg/24 h is highly efficient in severe Gram negative infections in the neonatal period If the serum concentration curve was carefully monitored there was no difficulty in calculating the adequate dose No apparent adverse reactions were registered during the neonatal period or at follow up examinations In comparison Klastersky et al (8) found only one case of temporarily decreased hearing with bilateral audiometric changes among 25 urological patients on sisomicin

In investigations on newborn infants one cannot avoid combination treatment in cases of highly suspected septicemia of unknown etiology In 15 cases sisomicin was combined with ampicillin in conventional doses In these cases nothing can be concluded about the efficacy of sisomicin per se However sisomicin was the only antibiotic in 9 cases with positive culture and the treatment resulted in complete cure

Our results agree well with observations by other authors Calderón Jaimes (3) using a sisomicin dosage of 3 mg/kg/24 h reported total remission of the infection in 20 out of 22 children below 6 years of age In his series only 7 patients were younger than five months of age Orzalesi (14) used sisomicin in doses up

to 7 5 mg/kg/24 h prophylactically in preterm infants Therefore the effect cannot be evaluated

The serum concentrations of sisomicin in our series were similar to those observed after gentamic n in the same age group (9 12) This refers both to peak values and the slope of the concentration curve

Serum half life for sisomicin in adults has been calculated to between two and four hours (11 19) From Figs 1 and 2 it is evident that half life estimates in our series often were higher It appeared that the higher values were not correlated to postnatal age whereas there was a high correlation to maturity and body weight respectively From Fig 1 it is clear that *T*/2 reached adult values at a gestional age of 40 weeks This observation corresponds well with investigations on gentamicin by McCracken et al (12) and Andersen et al (1) The validity of our estimates is substantiated by a calculation from Orzalesi s (14) data for *T*/2 and body weight The regression line derived from his series ($y=7\ 4-x$) is practically identical with $y=7\ 1-1\ 1x$ as calculated from our material From the clinical view point this implies that in administration of sisomicin to preterm and low birth weight infants the prolonged half life should be taken into consideration and the dose interval accordingly adjusted

The relation of *T*/2 to the age of gestation is striking It is probably an effect of renal immaturity at birth (17) Mosegaard et al (13) in a group of 7 adult patients with decreased renal function (creatinine clearance 18±1 7 ml/min) did show conspicious increase of serum half life of sisomicin

In vitro investigations in several laboratories (6 18 20) have shown that sisomicin is more efficient than other antibiotics against e g *Klebsiella Enterobacter E coli Proteus* and *Streptococcus pyogenes* The previously reported clinical series (2 8 13 19) have shown a satisfactory clinical efficacy with a low rate of adverse reactions This series of infections in the neonatal period agrees well with these results Thus sisomicin must be considered a valuable therapeutic alternative in the treatment of serious neonatal infections

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Table 3 *Average serum concentration of sisomicin in neonates*

	P_0		P_{1h}		P_{3h}		P_{6h}	
Single dose	Mean	S D	Mean	S D	Mean	S D	Mean	S D
≤1.75 mg/kg	1.21	0.94	4.42	1.49	3.23	1.48	1.57	0.76
≥1.75 mg/kg	1.44	0.35	4.03	1.66	3.03	1.37	1.97	0.97

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The average serum concentrations of sisomicin after a single dose are given in Table 3 Since clinical cure was complete in 90% of the cases it was not possible to correlate the clinical outcome to individual serum concentrations

DISCUSSION

It has been shown in this study that sisomicin in doses of 2.8–6.6 mg/kg/24 h is highly efficient in severe Gram negative infections in the neonatal period If the serum concentration curve was carefully monitored there was no difficulty in calculating the adequate dose No apparent adverse reactions were registered during the neonatal period or at follow up examinations In comparison Klastersky et al (8) found only one case of temporarily decreased hearing with bilateral audiometric changes among 25 urological patients on sisomicin

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Submitted June 24 1976
Accepted Nov 10 1976

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Acta Pædiatr Scand 66 323–327 1977

CONGENITAL RICKETS

Study of the Evolution of Secondary Hyperparathyroidism

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From the Service de Neonatologie du Professeur Maurice Bethenod et unite INSERM U 34 Hopital Debrousse Lyon France

ABSTRACT Sann L David L Frederich A Bovier Lapierre M Bourgeois J Romand Monier M and Bethenod M (service de Neonatologie du Professeur Maurice Bethenod et Unité Inserm U 34 Hopital Debrousse Lyon France) Congenital rickets Study of the evolution of secondary hyperparathyroidism Acta Paediatr Scand 66 323 1977.—A case of congenital rickets of nutritional origin is described in a light for-date premature infant (gestational age 34 weeks birthweight 1100 g) X rays of the long bones showed spread frayed and cupped metaphyses at birth and at the age of 16 days Serum calcium was 8.2 mg/100 ml phosphorus 3 4 mg/100 ml and alkaline phosphatase (A P) 323 IU/ml (N≤200) at the age of 3 days Very high level of serum immunoreactive parathyroid hormone (iPTH) was found at the age of 16 days 295 μlEq/ml (N≤50) Evidence of maternal vitamin D deficiency was demonstrated by low plasma 25 hydroxycholecalciferol (25-OH CC) 1 0 ng/ml (N 13.2±4 2) soon after delivery it was found to be normal (10.2 ng/ml) six months later Ca infusion (15 mg/kg/3 h) resulted in a marked fall of serum iPTH (280 to 84 μlEq/ml) Administration of vitamin D_2 (2400 IU/day for 10 days) induced some healing of the metaphyses A P remained elevated (400 IU/ml) plasma 25 OH CC was normal 10 2 ng/ml and serum iPTH was 115 μlEq/ml When 25 OH CC was given orally for ten days (15 μg/day) plasma 25-OH-CC rose to 64 5 ng/ml with a minor change of serum iPTH (94 μlEq/ml) X rays of the bones showed osteoporosis These results suggest a reduced conversion of 25-OH CC into 1 25-(OH)$_2$-CC

KEY WORDS Congenital rickets parathyroid hormone 25 hydroxycholecalciferol secondary hyperparathyroidism

Infantile rickets is a well known disease which still occurs Late rickets has been described in adolescent populations (12) but few neonatal rickets have been reported in the medical literature We describe a new case in which the evolution was studied by determinations of serum immunoreactive parathyroid hormone (iPTH) concentrations

CASE REPORT

The mother was a 4-year-old Caucasian who never complained of gastrointestinal symptoms or of any pain in the bones Her first pregnancy was complicated by toxemia and premature delivery at seven months of a male infant weighing 1880 g who died from intracranial bleeding at the age of ten days During the second pregnancy mild toxemia was detected therefore the mother was put on a salt restricted diet at 25 weeks of pregnancy Her daily intake of vitamin D at that time was estimated from dietary recall to be approximately 150 I U She remained in bed at home until the 34th week when a cesarean section was performed Twenty days after delivery she was found to have normal serum calcium (Ca) 10 0 mg/100 ml phosphorus (P) 4 4 mg/100 ml and alkaline phosphotases (A P) 76 IU/ml X ray films of the skeleton did not show osteomalacia Blood carotene 235 μg/100 ml (N>100) blood folate 97 6 ng/ml (N=1 9±35) and serum folate 5 1 ng/ml (N 9 ?±4 ?) were also found to be normal She was delivered of a female infant on February 7th 1975 Apgar score 8/10 The birthweight 1100 g length 39 5 cm and head circumference 28 5 cm were all under the tenth

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Table 1 *Evolution of the biochemical data*

Serum calcium (Ca) phosphorus (P) and parathormone (iPTH) levels plasma 25 hydroxycholecalciferol (25 OH-CC) *Calcium infusion was performed* with 15 mg/kg of calcium as calcium gluconate for three hours O basal values 3 h=values at the end of infusion R=Rickets O=osteoporosis N=normal

Age												
Days	2	3 → 12	16	22		25→36	39→48	53→64	70	77		
Months						1		2			3	10
Therapy Vit D (IU/d)	250	250	500	Ca inf 0	+3h	2 400	25 OH-CC administ 15 μg/d	2 400	600 000			1 600
Serum												
Ca (mg/100 ml)	8 2	9 1	8 9	9 2	9 6	9.5	9 0	9 8		10 3	10 3	10 2
P (mg/100 ml)	3 4	3 4	3 0	6 0	6 6	5 8	6 5	6 0		6 2	–	5 2
A P (IU/ml)		3?3	332	400		466	470	58?		443	–	380
iPTH (μlEq/ml) (N<50)			280	?80	86	115	94	78		–	42 5	45
Plasma 25 OH-CC (N=13 2±4 4 ng/ml)			9 4			10 8	64.5			36 6	15 6	20 4
X rays (legs)	R		R			0	0	0			N	N

One load dose

parathyroid serum was used as a standard reference Normal range in children and adults never exceeded 50 microliters equivalent per milliliter (μlEq/ml) Approximately 90% of normal subjects have detectable levels of serum iPTH (3)

Plasma 25 hydroxycholecalciferol (25-OH CC) was measured by a *competitive protein* binding method modified from the assay of Preece et al (16) Normal values in infants are 13 3±4 4 ng/ml Calcium infusions were *performed for three hours* with 15 mg/kg of calcium as calcium gluconate The effect of oral administration of 25 hydroxycholecalciferol (15 μg/day for ten days) on plasma 25 OH CC and serum iPTH levels was also studied

RESULTS

Twenty days after delivery, the mother's serum Ca was 10 0 mg/100 ml P 4 4 mg/100 ml A P 76 IU/ml iPTH 35 μlEq/ml plasma 25 OH-CC level was particularly low 1 0 ng/ml Six months later she had normal plasma 25 OH CC (11 0 ng/ml)

The data from her infant are shown in Table 1 Elevated serum iPTH level was detected at the age of 16 days Plasma 25-OH CC measured after administration of small doses of vitamin D_2 was found to be at the lower range of normal values Calcium infusion resulted in minimal change in serum Ca but a marked

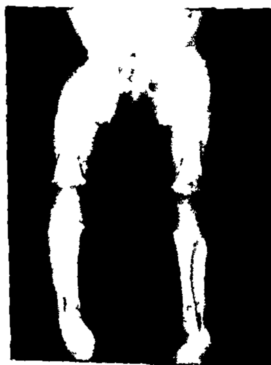


Fig 3 Roentgenogram of the legs at the age of 48 days after treatment with 15 μg 25 hydroxycholecalciferol daily for ten days No more features of rickets is observed *Shafts are* osteoporotic with marked thinning of the cortex



Fig 1 Roentgenogram of the abdomen at the age of 3 days There is a marked spread metaphysis at the distal end of the femur

percentile of Lubchenco s growth curve (11) On the second day of life she was admitted to the Hopital Debrousse for pneumoperitoneum and pneumothorax which were cured by exsufflation and oxygen therapy Initial laboratory tests showed 3610000 erythrocytes per mm³ with 15 2 g/100 ml hemoglobin 8400 white cells with 86% neutrophils Serum electrolytes were normal sodium 133 mEq/l potassium 5 2 mEq/l bicarbonates 19 5 mEq/l chloride 102 mEq/l Blood glucose was 35 mg/100 ml serum protein 4 7 g/100 ml blood urea nitrogen 18 mg/100 ml

Subsequent examination showed mildly separated sutures with normal skull bones There was no swelling at the wrists or ankles and the end of the ribs were not enlarged Serum Ca was 8 2 mg/100 ml magnesium 2 3 mg/100 ml P 3 4 mg/100 ml High alkaline phosphatase was found (323 N~150 IU/ml)

Rickets was suspected on X rays on the second day (Fig 1) and confirmed at sixteen days with spread and frayed metaphyses on the roentgenogram of the legs (Fig 2) Further studies showed urinary Ca excretion between 0 3 and 1 43 mg/kg/day and urinary P excretion between 22 and 31 2 mg/kg/day Urinary amino acids chromatography showed no abnormality The infant received 250 to 500 IU per day of vitamin D_2 I V from the second to the ninth day of life Then she was given 2400 IU/day of vitamin D_2 After administration of 600000 IU of vitamin D_2 she was discharged from the hospital at the age of eighty days At the age of three months the infant weighed 3420 g (−2 S D) length 50 cm (−2 S D) and normal head circumference (38 cm) Clinical examination and roentgenogram of the bones were satisfactory At the age of 13 months her weight was 8270 g (−1 S D) the length 75 cm (+ 1 S D) and the head circumference 46 cm (+1 S D) Clinical examination was normal and revealed four normal teeth Roentgenogram of the long bones was also normal The evolution of the biochemical data is shown in Table I

SPECIAL STUDIES

Methods

The infant was initially infused with 10% dextrose 60 to 120 ml/kg/24 h Then she was fed a commercial humanized milk providing 67 calories/100 ml with calcium 57 0 mg/100 ml and phosphorus 44 0 mg/100 ml Feeding was started at the age of nine days with 110 ml/kg/day then 175 ml/kg/day were given at the age of 21 days 200 ml/kg/day at the age of 54 days and 250 ml/kg/day the age of 60 days

Serum Ca was determined by atomic absorption and serum P according to Fiske & Subbarow (6)

Serum immunoreactive parathyroid hormone (iPTH) was measured by radioimmunoassay using an antibovine parathyroid hormone guinea pig antiserum (GP 6) a gift of Dr Constantine Anast from the Department of Pediatrics University of Missouri Hyper



Fig 2 Roentgenogram of the legs at the age of 16 days The metaphyses of the femurs are spread frayed and irregularly mineralized

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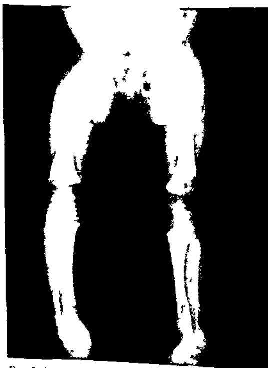


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When 2400 units of vitamin D_2 were given for 10 days, a normal level of plasma 25 OH CC was found a decrease in serum iPTH was observed but a normal level was not obtained Roentgenogram of the legs showed no more features of rickets When 15 µg/day of 25 OH CC was given orally for 10 days plasma level of 25 OH CC increased six fold while the decrease in serum iPTH was only 16 5% Roentgenogram of the legs showed osteoporosis at the end of 25 OH CC administration (Fig 3)

DISCUSSION

All the biochemical and radiological features found in this infant agree with the diagnosis of congenital rickets

Congenital rickets has been a well known disease since the 1914–18 war Begum et al (2) demonstrated its occurrence in newborn infants whose mothers were suffering from coeliac disease However although intestinal biopsy was not performed in the mother of this child this diagnosis was made unlikely by the absence of any clinical symptoms and by the normal concentrations of blood carotene and folate after delivery The vitamin D intake of the mother was low during the second trimester of her pregnancy furthermore she was not exposed to sunlight Therefore it is likely that the neonatal rickets was nutritional in origin This is further supported by the finding of very low level of plasma 25 OH CC in the mother soon after birth and by its spontaneous normalization 6 months later

Congenital rickets of nutritional origin has recently been described by Ford et al in Asian immigrants in Great Britain (7) Moncrieff & Fadahunsi (13) reported a similar case with low levels of plasma 25 OH CC in the mother and the newborn infant This is however the first report demonstrating the secondary hyperparathyroidism in congenital rickets by iPTH determinations According to Lamb & Stanbury (10) a marked decrease in serum iPTH under Ca infusion indicates that hyperparathyroidism is not autonomous In the patient persistant hyperparathyroidism was found despite normal levels of serum Ca and plasma 25 OH CC Moreover, when 25 OH CC was given orally plasma 25 OH CC increased six fold hence excluding intestinal malabsorption Serum iPTH fall was however small (less than 20%) and it remained far above the normal range

A direct suppressive effect of vitamin D on iPTH by the parathyroid cells has been demonstrated (10 15) and evidence for such an effect in a case of congenital hyperparathyroidism secondary to maternal hypoparathyroidism has been reported (18) Recently Chertow et al (4) attributed this effect to 1 25 dihydro xycholecalciferol (1 25 (OH) CC) since administration of physiological doses of 1 25 $(OH)_2$ CC to rachitic rats resulted in a 40% decrease in serum PTH The minor change in serum PTH which occurred in spite of marked increase in plasma 25 OH CC level observed in this infant suggests a limitation in conversion of 25 OH CC into 1 25 OH CC

Osteoporosis is a common finding in tiny premature infants It is usually attributed to the low Ca intake in comparison to the amount a fetus receives from its mother (5 19) The diet given to our infant did not provide daily amounts of calcium comparable to the theoretical values and this could explain the occurrence of osteoporosis Intestinal Ca absorption is also poor in premature infants (1 8 21) it does not depend solely on fat malabsorption and it increases with fetal and post natal age Furthermore it is not adapted to the requirements and the body stores of calcium as in children and adults (5) 1 25 $(OH)_2$ CC plays a major role in intestinal Ca absorption (15) and it has been shown that its serum level increases with calcium restricted diets (9) In this case osteoporosis was observed despite 25 OH CC administration and elevated plasma 25 OH CC levels It is suggested that reduced

1 hydroxylation may be one of the factors responsible for Ca malabsorption in premature infants and the need for large calcium supplementation

It is speculated that the small amount of 1 25-$(OH)_2$-CC produced by the premature infant could probably have healed the rickets but was insufficient to stimulate the intestinal factors (Ca binding protein (8) and/or Ca ATPase (17)) involved in Ca absorption as it was unable to correct the secondary hyperparathyroidism

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DISCUSSION

All the biochemical and radiological features found in this infant agree with the diagnosis of congenital rickets

Congenital rickets has been a well known disease since the 1914–18 war Begum et al (2) demonstrated its occurrence in newborn infants whose mothers were suffering from coeliac disease However although intestinal biopsy was not performed in the mother of this child this diagnosis was made unlikely by the absence of any clinical symptoms and by the normal concentrations of blood carotene and folate after delivery The vitamin D intake of the mother was low during the second trimester of her pregnancy furthermore she was not exposed to sunlight Therefore it is likely that the neonatal rickets was nutritional in origin This is further supported by the finding of very low level of plasma 25 OH CC in the mother soon after birth and by its spontaneous normalization 6 months later

Congenital rickets of nutritional origin has recently been described by Ford et al in Asian immigrants in Great Britain (7) Moncrieff & Fadahunsi (13) reported a similar case with low levels of plasma 25 OH CC in the mother and the newborn infant This is however the first report demonstrating the secondary hyperparathyroidism in congenital rickets by iPTH determinations According to Lamb & Stanbury (10) a marked decrease in serum iPTH under Ca infusion indicates that hyperparathyroidism is not autonomous In the patient persistant hyperparathyroidism was found despite normal levels of serum Ca and plasma 25 OH CC Moreover, when 25 OH CC was given orally plasma 25 OH CC increased six fold hence excluding intestinal malabsorption Serum iPTH fall was however small (less than 20%) and it remained far above the normal range

A direct suppressive effect of vitamin D on iPTH by the parathyroid cells has been demonstrated (10 15) and evidence for such an effect in a case of congenital hyperparathyroidism secondary to maternal hypoparathyroidism has been reported (18) Recently Chertow et al (4) attributed this effect to 1-25 dihydroxycholecalciferol (1 25 $(OH)_2$ CC) since administration of physiological doses of 1-25 $(OH)_2$ CC to rachitic rats resulted in a 40% decrease in serum PTH The minor change in serum PTH which occurred in spite of marked increase in plasma 25 OH CC level observed in this infant suggests a limitation in conversion of 25 OH CC into 1 25 OH CC

Osteoporosis is a common finding in tiny premature infants It is usually attributed to the low Ca intake in comparison to the amount a fetus receives from its mother (5 19) The diet given to our infant did not provide daily amounts of calcium comparable to the theoretical values and this could explain the occurrence of osteoporosis Intestinal Ca absorption is also poor in premature infants (1 8 21) it does not depend solely on fat malabsorption and it increases with fetal and post natal age Furthermore it is not adapted to the requirements and the body stores of calcium as in children and adults (5) 1 25 $(OH)_2$ CC plays a major role in intestinal Ca absorption (15) and it has been shown that its serum level increases with calcium restricted diets (9) In this case osteoporosis was observed despite 25 OH CC administration and elevated plasma 25 OH CC levels It is suggested that reduced

1 hydroxylation may be one of the factors responsible for Ca malabsorption in premature infants and the need for large calcium supplementation

It is speculated that the small amount of 1 25 $(OH)_2$ CC produced by the premature infant could probably have healed the rickets but was insufficient to stimulate the intestinal factors (Ca binding protein (8) and/or Ca ATPase (17)) involved in Ca absorption as it was unable to correct the secondary hyperparathyroidism

ACKNOWLEDGEMENT

We have the great pleasure to thank Dr Donald Barltrop for reviewing the manuscript

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Submitted July 15 1976
Accepted Oct 22 1976

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SEVERE DISSEMINATED ADENOVIRUS INFECTION SUCCESSFULLY TREATED WITH A THYMIC HUMORAL FACTOR THF

I VARSANO T M SCHONFELD Y MATOTH B SHOHAT T ENGLANDER V ROTTER and N TRAININ

From the Department of Pediatrics and the Clinical Laboratory Beilinson Medical Center Tel Aviv University Medical School Petah Tikva and the Department of Cell Biology the Weizmann Institute of Science Rehovot Israel

ABSTRACT Varsano I Schonfeld M Matoth Y Shohat B Englander T Rotter V and Trainin N (Department of Pediatrics and the Clinical Laboratory Beilinson Medical Center Tel Aviv University Medical School Petah Tikva and Department of Cell Biology the Weizmann Institute of Science Rehovot Israel) Severe disseminated adenovirus infection successfully treated with a thymic humoral factor THF Acta Paediatr Scand 66 329 1977.—Adenovirus and other usually benign viral infections may occasionally be associated with severe fulminant disease often accompanied by acute acquired cellular immunodeficiency Thymic humoral factor derived from calf thymuses has been demonstrated to have the capacity to restore the immunocompetence of immature incompetent T cells This factor was used in the treatment of a 3½ year-old boy who was critically ill with an adenovirus infection and presented evidence of immunocellular deficiency Within less than 48 hours after the institution of treatment with thymic humoral factor there was a dramatic progressive clinical improvement with restoration of the cellular immunocompetence It is suggested that thymic humoral factor may be beneficial in the treatment of severe viral infections associated with depressed cellular immunocompetence

KEY WORDS adenovirus infection viral infection thymic humoral factor cellular immunodeficiency

Infection with adenovirus which is usually associated with mild febrile conditions (14) occasionally develops into a severe disseminated illness (1 9 15) It has been suggested that in such patients the fulminant course of the disease may be due to a deficiency in the defense mechanism of the host (2 3)

Thymic humoral factor (THF) is a polypeptide with hormone like activity which has been isolated recently from calf thymuses (4 16) In vitro and in vivo studies performed both in animals and in humans have shown that THF and other thymic factors have the capacity to enhance and restore the cellular immune response by inducing maturation of T lymphocytes (6 11 16 18 19) Up till the present time the clinical use of these thymic factors has been very limited (18 19)

The present report concerns a child with severe disseminated adenovirus infection and transient depression of the cell mediated immunocompetence (CMI) who made a remarkable recovery with reconstitution of the CMI following the administration of THF

CASE REPORT

A 3½ year-old boy was hospitalised because of acute respiratory infection of 4 days duration The child had been hospitalised repeatedly for the same reason since the age of one month No congenital abnormality of the respiratory tract was found and the levels of serum immuno-

globulins and of sweat electrolytes were normal On admission his temperature was 40 5 C There was severe respiratory distress with clinical and radiological evidence of diffuse bilateral interstitial pneumonia The liver and spleen were palpable 4 and 3 cm respectively below the costal margin The white blood cell count was 7 1 10^9/l with a shift to the left and lymphopenia of 13% (0 923 10^9/l) LDH was 10 58 μmol s^{-1}/l SGOT 1 151 μmol s^{-1}/l prothrombin 0 35/l total proteins 63 g/l albumin 35 g/l IgG 8 0 arb unit IgA 1 8 arb unit IgM 0 78 arb unit Secretory IgA was present in the saliva Routine urine examinations were normal

Evaluation of the cellular immunocompetence showed total T-cells (as tested by the E rosette test) 0 22 10^9/l (normal values in our laboratory for children in this age group are 1 4±0 3 10^9/l±S D) migratory inhibition factor (MIF) index of 0 95 and 0 9 with PPD and Concanavalin A respectively (standard MIF ≤0 8) (10) and negative skin tests to antigens of Candida at a dilution of 1 1000 Trychophyton 1 1000 PPD 2 tuberculin units and streptokinase streptodornase 1 1

Despite 4 days of intensive supportive therapy including cephalexin and prednisone (2 mg/kg/d) the child s clinical condition deteriorated further Melena appeared and he became comatose with depressed spontaneous respiration The child was intubated and mechanical ventilation instituted The cerebrospinal fluid was normal Electroencephalogram tracing revealed a diffuse slowing of the cortical activity There was no evidence of disseminated intravascular coagulation Serum glucose and ammonia were normal During the following 3 days the child s condition continued to deteriorate Chest X rays demonstrated extension of the pulmonic infiltrates and the child remained in deep coma

A fulminant viral disease was suspected and from the seventh day of hospitalisation THF was administered at a dose of 2 mg/kg/d intramuscularly with the aim of restoring the impaired CMI At the same time prednisone was tapered off Within 24 hours a dramatic clinical improvement was observed manifested by the appearance of spontaneous breathing and adequate oxygenation of the blood after discontinuation of the mechanical ventilation One day later the child s temperature fell to normal and he regained consciousness Seven days following institution of THF treatment there was a significant reduction in respiratory distress and partial resolution of the interstitial findings in the lungs The electroencephalographic pattern had also returned to normal THF was discontinued after 2 weeks of administration and 7 days later the child was discharged with minimal residual lung findings

Re-evaluation of the CMI following THF administration revealed an increase in the absolute number of T cells to 1 38 10^9/l and 1 78 10^9/l on the third and fifth days of treatment respectively This increase was maintained in 5 additional examinations made during the following 11 months The MIF index against PPD and Concanavalin A was normal (0 80 and 0 78 respectively) on the tenth day of treatment as well as on two later occasions Skin tests repeated after 5 weeks showed a positive reaction to streptokinase streptodornase (12 mm induration and 18 mm erythema) as they did after 5 months

Infection with type 3 adenovirus was diagnosed based on the isolation of the virus from tracheal aspirate and a rise in titer of complement fixing antibodies from 1 16 on the fifth day of hospitalisation to 1 256 on the 17th day

DISCUSSION

In the patient described severe interstitial pneumonia encephalopathy and hepatic damage resulting from the adenovirus infection were associated with impaired CMI which most probably was acquired during the illness The reconstitution of CMI following the administration of THF was associated with a strikingly rapid clinical improvement unusual in the course of such a fulminant disease (1 15)

In patients with disseminated adenovirus infections the humoral immunity is usually normal (12 13) although severe lymphopenia (<1 5 10^9/l) is common (1–3) Transient lymphopenia and a decrease in the number of T cells have also been reported in other viral infections (2 7 11 17) The finding of normal lymphatic tissue in such patients suggests an acute acquired form of cellular immunodeficiency (1) Severe adenovirus infection has been reported in patients with thymic alymphoplasia (20) as well as in other conditions associated with an impaired CMI e g in malnourished children (5) and following measles (12)

The importance of intact cellular immunoresponsiveness for recovery from viral infections is well known (8) The striking clinical improvement concomitant with restoration of the CMI which was observed in our patient can almost certainly be related to the THF administered particularly since this and other thymic factors have already been shown to have the capacity to restore CMI in various pathological conditions (4 18 19) including viral infections in humans (11)

Although no definite conclusions can be drawn on the basis of a single case it is suggested that therapy with THF may be beneficial in the treatment of life threatening viral infections associated with a depressed CMI

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Submitted July 19 1976
Accepted Dec 10 1976

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EFFECT OF MATERNAL PARITY AND INFANT SEX UPON THE HAEMATOLOGICAL VALUES OF CORD BLOOD

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ABSTRACT Lind T Gerrard J Sheridan T S and Walker W (MRC Reproduction and Growth Unit Princess Mary Maternity Hospital Newcastle upon Tyne and Department of Haematology Royal Victoria Infirmary Newcastle upon Tyne Northumberland UK) Effect of maternal parity and infant sex upon the haematological values of cord blood Acta Paed atr Scand 66 333 1977.—Coulter Counter (Model S) analysis of 400 cord blood samples are presented together with differential white cell counts for 249 of these The mean values for haemoglobin and red cell count are somewhat higher than previous values determined by manual methods while those for haematocrit and mean cell volume are slightly lower An unexpected finding was that the red cell count haemoglobin concentration and haematocrit are significantly higher in male than in female infants A parity effect was also demonstrated Infants of both sexes born as second or subsequent births had lower values for total white cell count haemoglobin concentration and haematocrit than first born infants

KEY WORDS Haemoglobin white blood count red blood count cord blood parity infant sex

Most reports on detailed analyses of human cord blood are over 20 years old (2 3 4 5 6 8 9) The values were no doubt determined with care and precision but by manual techniques which are much less accurate and less reproducible than automated methods (7)

A paper by Xanthou (10) reported the use of a Coulter counter to determine the total white cell count (WBC) in a group of healthy new born babies but did not report on other haematological indices We have not found any reports of full cord blood analysis in any large group of infants using modern analytical techniques It is the purpose of this paper to report data obtained by Coulter counter analysis of 400 umbilical venous blood samples together with the differential white cell counts in 249 of these

MATERIALS AND METHODS

Infants

Samples were obtained from over 400 virtually consecutive deliveries no attempts were made to select the type of patient or infant other than to exclude those infants known to be affected by rhesus haemolytic disease Because of the fragility of white cells under storage conditions it was often the case that samples collected from infants delivered between 12 00 noon on Saturdays and midnight on Sundays were unsuitable for white cell counting by the Monday morning Such samples were discarded as were any showing evidence of clot formation the survey was terminated on completion of 400 acceptable samples

Coulter analysis

Blood was obtained from an umbilical vein in the placental end of the cord immediately upon clamping This blood was put into a standard sequestrene tube and sent for analysis or kept at 4 C from overnight deliveries until collected for analysis Determinations were performed routinely on the Coulter equipment (Model S) used for the regular hospital service

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Acta Pædiatr Scand 66 333–337 1977

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Table 3 *Haematological values from babies requiring admission to the special care nursery (SCN) compared with the 276 'normal' babies used for Table 2 (Means and standard deviations)*

Haematological value	SCN babies	"Normal babies"
WBC×10 per litre	14 06±5 49 (40)	13 60±3 75 (276)
RBC×10 ¹² per litre		
Males	4 64±0 53 (22)	4 70±0 4 (137)
Females	4.59±0 37 (18)	4 56±0.37 (139)
Both sexes	4 67±0 44 (40)	4 63±0 40 (276)
Hb (g/dl)		
Males	16 74± 22 (22)	16 99±1 70 (137)
Females	16 89±1 51 (18)	16 41±1.37 (139)
Both sexes	16 81±1 91 (40)	16 0±1.57 (276)
HCT (l/l)		
Males	0 489±0 060 (22)	0 496±0 049(137)
Females	0 495±0 046 (18)	0 479±0 047(139)
Both sexes	0 49 ±0 053 (40)	0 488±0 046(276)
MCV (fl)	106 07±4 38 (40)	105 07±4 40 (276)
MCH (pg)	36 6±1 50 (40)	36 00±1 40 (276)
MCHC (g/dl)	34 08±1 00 (40)	34 19±1 03 (276)

22 4% at 41 weeks 5 2% at 42 weeks and 2 2% at 43 or more completed weeks

Method of delivery A normal vaginal delivery occurred in 79 8% by forceps in 14 2% an assisted breech delivery in 3 1% and by Caesarean section in 2 8% Some under representation of deliveries by Caesarean section was caused by lack of sufficient staff in theatre to take the collection of blood samples from all cases otherwise the figures are reasonably representative of the usual hospital annual experience

Numbers of samples Full blood counts were available on 400 samples and the percentage of reticulocytes was estimated for 316 of these differential white cell counts were available on 249 samples but 20 of these patients had uncertain menstrual dates

Sex related differences When the means and standard deviations for the various estimates were calculated it was found that male infants had a significantly higher red blood count (RBC) haemoglobin concentration (Hb) and haematocrit (Hct) than female infants but there was no significant difference for any of the other determinations (Table 1)

Centile values To make the data as representative of normal infants as possible the calculations were based upon the 276 infants delivered between 37 and 41 completed weeks of gestation who weighed over 2 5 kg and who did not require any special care at or after delivery The values are given in Table 2 and are for both sexes with the exception of RBC Hb and Hct where the sex specific values were calculated

Differential WBC There was a great variation in the percentage of neutrophils found in the 249 samples examined and it was ascertained that the neutrophil value was not correlated with the total WBC irrespective of the value of the latter ($r=0\ 049$) The percentage of neutrophils is very similar for all values of total WBC whether this be less than ten thousand per mm³ or over eighteen thousand On many occasions the neutrophil count was less than 45% again this was not related to the total WBC and occurred with equal incidence over the whole range of white cell counts It was quite sufficient to examine the percentage of neutrophils because the percentage of lymphocytes complemented it to 100% for all practical purposes

The data were examined in some detail to determine whether the haematological values were related in any way to fetal maternal or delivery characteristics

Admission to the intensive care nursery The most obvious group in whom changes might have been anticipated were those babies requiring special care immediately after delivery There were 40 such infants and their data were compared with the 276 infants described above No significant differences were found (Table 3) Their values were then distributed in relation to the centiles from the normal babies and again no significant differences were found

Birth weight Over the whole group no correlation could be demonstrated between birth

Table 1 *Means and standard deviations of the various estimates by infant sex (Occasions where males differ from females are indicated in the last column)*

Haematological value	Male infants	Female infants	Both	Sign $P<$
WBC×10⁹ per litre	13 36± 4 15 (203)	13 97± 3 78 (197)	13 66± 3 98 (400)	NS
RBC×10¹² per litre	4 69± 0 44	4 56± 0 39	4 63± 0 42	0 01
Hb (g/dl)	16 90± 1 77	16 48± 1 46	16 69± 1 63	0 01
HCT (l/l)	0 4935± 0 0500	0 4812± 0 0437	0 4874± 0 0474	0 01
MCV (fl)	104 94± 4 41	105 21± 4.53	105 07± 4 47	NS
MCH (pg)	35 93± 1 47	36 06± 1 44	36 00± 1 46	NS
MCHC (g/dl)	34 17± 1 02	34 20± 1 02	34 18± 1 02	NS
Reticulocytes (%)	6 03± 1 76 (166)	5 99± 1 76 (150)	6 01± 1 77 (316)	NS
Neutrophils (%)	61 22±17 84 (129)	62 52±16 42 (120)	61 85±17 15 (249)	NS
Lymphocytes (%)	34 07±16 33	32 56±14 78	33 34±15 59	NS
Monocytes (%)	2 89± 1 94	3 40± 2 33	3 14± 2 15	NS
Eosinophils (%)	1 82± 1 83	1 52± 1 59	1 67± 1 72	NS

Differential counts

It was not possible to undertake a differential count in every case and out of 400 cases in which white cell counts were obtained differential counts were made in only 249 After staining with Leishmann stain two independent technicians each counted two groups of 100 cells and the mean of these four counts was reported twenty five slides were recounted by the same technicians under blind conditions and 20 slides randomly chosen from the remainder were counted by an independent observer While there were differences in the differential counts none of these was significant and the data reported here are the original findings

RESULTS

Infant sex and birthweight There were 203 male infants with a mean birth weight of 3 40±0 50 kg and 197 females with a mean birth weight of 3 25±0 46 kg Calculating from the first day of the last menstrual period in the 362 patients who had reliable dates 1 1% were delivered at 33 completed weeks or less 3 9% at 34 or 35 weeks 3 6% at 36 weeks 3 6% at 37 weeks 58 0% at 38–40 weeks,

Table 2 *Centile values of the various estimates for babies delivered between 37 and 41 completed weeks of gestation weighing over 2 5 kg and not admitted to the special care nursery (276 infants both sexes unless otherwise indicated)*

	Centiles						
Haematological value	3	10	25	50	75	90	97
WBC×10⁹ per litre	6 88	9 76	11 15	13 38	15 65	18 54	22 57
RBC×10¹² per litre							
Males	4 02	4 23	4 45	4 73	4 98	5 23	5 50
Females	3 93	4 08	4 25	4 59	4 83	5 07	5 27
Both sexes	3 97	4 13	4 35	4 66	4 90	5 14	5 38
Hb (g/dl)							
Males	14 13	15 08	16 01	17 07	18 03	19 11	20 29
Females	14 14	14 73	15 40	16 44	17 44	18 34	19 07
Both sexes	14 14	14 81	15 64	16 78	17 79	18 68	19 79
HCT (l/l)							
Males	0 411	0 441	0 465	0 499	0 526	0 556	0 589
Females	0 410	0 428	0 447	0 478	0 514	0 533	0 554
Both sexes	0 410	0 432	0 455	0 489	0 521	0 544	0 579
MCV (fl)	98 0	100 2	102 6	105 0	108 5	111 2	114 0
MCH (pg)	33 5	34 2	35 0	35 9	37 0	38 0	38 8
MCHC (g/dl)	32 1	32 9	33 6	34 3	34 9	35 5	36 4
Reticulocytes (%)	3	4	5	6	7	8	9
Neutrophils (%)	20	39	52	65	75	83	87
Lymphocytes (%)	10	15	22	32	42	56	70

globin and red cell count are somewhat higher than previously found while those for haematocrit and mean cell volume are slightly lower (2 3 4 5 6 8 9) The haematocrit change is explicable on the grounds that trapped plasma is not included with a Coulter analysis but the association with a lower MCV was unexpected

We have not found any report that describes an effect of fetal sex upon the red cell count haemoglobin concentration or haematocrit It has been argued that the lower haemoglobin concentration found in women during their childbearing years is due to the iron loss caused by menstruation or to the iron demands of pregnancy This hypothesis is apparently supported by the fact that the haemoglobin concentration rises after the menopause However an alternative suggestion could be that the hormonal status of women particularly their sex steroid status over their fertile years leads to altered haemopoiesis and that the lower haemoglobin concentration is the physiological norm for that period of their life (1) Certainly during pregnancy a time of considerable oestrogen and progesterone increase the maternal haemoglobin concentration decreases It can thus be postulated that the female fetus may respond to the hormonal environment of pregnancy in the same way that she will respond to her own hormonal change after puberty

It is less easy to think of a plausible explanation for the parity effect It cannot be explained by infants of greater birth weight labours of shorter duration the method of delivery or the length of time the membranes have been ruptured Whatever the origin of this effect it applies to male and female fetuses alike

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Submitted May 6 1976
Accepted Nov 2 1976

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Table 4. *Effect of parity and infant sex upon WBC, RBC, Hb and HCT. (Means and standard deviations)*

	Parity 0	*Parity 1+*	*Both*	Significance of parity *diff*
WBC × 10^9 per litre				
Males	13.92±4.01 (52)	12.70±3.47 (93)	13.14±3.71 (145)	
Females	14.47±3.86 (52)	13.43±3.54 (97)	13.79±3.69 (149)	
Both sexes	14.20±3.93 (104)	13.07±3.52 (190)	13.47±3.70 (294)	$p<0.05$
RBC × 10^{12} per litre				
Males	4.75±0.40	4.62±0.41	4.67±0.41	
Females	4.65±0.36	4.53±0.41	4.57±0.40	
Both sexes	4.70±0.38	4.57±0.41	4.62±0.41	$p<0.01$
Hb (g/dl)				
Males	17.17±1.47	16.59±1.67	16.80±1.62	
Females	16.73±1.29	16.32±1.55	16.46±1.47	
Both sexes	16.95±1.40	16.45±1.61	16.63±1.55	$p<0.01$
HCT (l/l)				
Males	0.5014±0.0463	0.4853±0.0477	0.4911±0.0477	
Females	0.4885±0.0425	0.4760±0.0445	0.4804±0.0441	
Both sexes	0.4950±0.0447	0.4806±0.0462	0.4857±0.0461	$p<0.01$

weight and any of the haematological variables.

Length of gestation. No correlations could be found between the cord blood values and length of gestation but there were few babies below 36 completed weeks.

Onset of labour. The data derived from babies born after a spontaneous onset of labour differed in no respect from those born following induction of labour. The mothers were then divided into three groups according to the time elapsing from the membranes rupturing to the infant being born (under 6 hours, 6–9 hours and over 9 hours); no significant differences were found for any of the haematological variables.

Method of delivery. The haematological findings were the same for babies born as spontaneous cephalic deliveries, with the use of forceps, by breech presentation or by Caesarean section with one exception. The mean white cell count was significantly higher (at the 5% level) in babies delivered by the use of forceps compared with the other three groups. However, 90% of the patients so delivered were primigravidae and raised the possibility that the difference was due to parity rather than the method of delivery. To test this the white cell count of forceps delivered infants of primigravid patients was compared with the counts of normally delivered infants of primigravid patients; no significant differences were found.

Parity. To determine whether parity had any effect only those women having normal vaginal deliveries and whose babies did not need to go to the special care nursery were considered. This of course reduced the number available for study (294) but made a comparison between the parity groups cleaner. The data are given in Table 4 and demonstrate that the values for white cell count, red cell count, haemoglobin and haematocrit are significantly greater for first born infants than for second or subsequent births; the sex differences are also maintained within each group. The other haematological variables did not show a parity difference.

CONCLUSIONS

Comparisons of our findings with previously reported values are affected by differences of methodology. Our mean values for haemo

globin and red cell count are somewhat higher than previously found while those for haematocrit and mean cell volume are slightly lower (2 3 4 5 6 8 9) The haematocrit change is explicable on the grounds that trapped plasma is not included with a Coulter analysis but the association with a lower MCV was unexpected

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Acta Pædiatr Scand 66 339-344 1977

ON INDICATIONS FOR TREATMENT OF THE HYPERPHENYLALANINEMIC NEONATE

FLEMMING GUTTLER and ERIK WAMBERG

From the John F Kennedy Institute Glostrup Denmark

ABSTRACT Guttler F and Wamberg E (The John F Kennedy Institute DK 2600 Glostrup Denmark) On indications for treatment of the hyperphenylalaninemic neonate Acta Paediatr Scand 66 339 1977.—Of 488 006 neonates tested by Guthrie screening 58 showed values above 2.5 mg/100 ml Thirty two showed values between 2 5 mg/100 ml and 15 mg/100 ml Eighteen of these infants appeared to have phenylketonuria (PKU) and fourteen to have persistent hyperphenylalaninemia (HPA) Neither the initial Guthrie test value nor the confirmatory test were able to differentiate between these two conditions. Consequently a phenylalanine restricted diet is started in any child with serumphenylalanine values exceeding 10 mg/100 ml (605 μmol/l) The data show that the course of the dietary tolerance of phenylalanine and a 24-hour phenylalanine load test will differentiate infants with PKU from those with HPA

KEY WORDS Neonatal hyperphenylalaninemia phenylketonuria phenylalanine tolerance indications for treatment

Blood amino acid screening of newborn babies has revealed heterogeneity in disorders related to the hydroxylation of phenylalanine to tyrosine (8 17 18) Early blood screening has enabled us to correct these metabolic disorders within the first few weeks of life (5 13 20) However the improvement in early detection and treatment of newborn children with elevated blood phenylalanine has hampered our possibilities of distinguishing those who require a phenylalanine restrictive dietary therapy e g children with phenylketonuria (PKU) from those who do not e g children with persistent hyperphenylalaninemia (HPA) according to previous diagnostic criteria such as the appearance of a positive ferric chloride reaction (7)

The present data show that initial screening values as well as serum phenylalanine and tyrosine concentrations in confirmatory fluorimetric tests may be misleading Thus seven babies who later appeared to have phenylketonuria showed confirmatory serum phenylalanine values in between 10 and 15 mg/100 ml (605–910 μmol/l) between the 9th and 22nd days of life Consequently a phenylalanine restricted diet is started in any child with serum phenylalanine values exceeding 10 mg/100 ml (605 μmol/l) Weekly estimates of the dietary tolerance of phenylalanine and a 24 hour phenylalanine load test at 6 months of age are used as the criteria for the differentiation of children with PKU from those with HPA

MATERIAL AND METHODS

This study includes 58 infants seen in Denmark with a positive Guthrie screening test (9) over a 9-year period (1967–1975) The Guthrie screening in Denmark is centralized in one laboratory and usually performed at the age of 5 to 7 days When a presumptive positive result was observed the phenylalanine and tyrosine concentration in serum were examined by a fluorimetric assay (see below) in additional samples of blood taken 4 to 15 days later Guthrie testing covered 0 1% newborn babies in Den

Table 1 *Blood phenylalanine related to diagnosis in 58 of 488 006 neonates with elevated Guthrie screening test at 5th to 7th days of life*

Diagnosis	Sex	Blood phenylalanine (mg/100 ml) 2 5–10	>10–15	>15
Hyperphenyl	♂	8	1	0
alaninemia	♀	5	0	0
Phenylketonuria	♂	4	4	10
	♀	4	6	16
Total		21	11	26

mark in 1967 increasing to 77 7% of all newborns in 1971 and 99 5% of the newborns in 1975

Phenylalanine was determined by an adaptation (10) of the fluorimetric method of McCaman & Robins (16) using 25 µl of serum The accuracy of the method was tested by comparing the values obtained by fluorimetry to column chromatography of 20 samples ranging from 95–665 µmol/l The correlation coefficient was 0 93

Tyrosine was determined by a microadaptation of the fluorimetric method of Udenfriend (19) using 150 µl of serum (10) Twenty two samples measured by this method and by column chromatography showed a correlation coefficient of 0 88 Serum standards with known amounts of phenylalanine and tyrosine were used in the fluorimetric assays in place of aqueous solutions of amino acids

The phenylalanine tolerance of a child was calculated weekly as the amount of phenylalanine in mmoles per kg body weight tolerated per day in order to keep the serum phenylalanine level within 180–425 µmol/l (3–7 mg/100 ml) The protein intake was kept at 13% of the intake of calories and the part of protein tolerated as natural protein was determined by the actual serum phenylalanine value of the child

For oral phenylalanine loading L phenylalanine (BDH Chemicals Ltd Poole England) was completely dissolved in 0 01 N HCl and the pure solution was given orally after an overnight fast in a dose of 0 6 mmol per kg body weight Phenylalanine loading performed according to this practice revealed maximum serum phenylalanine concentrations within the first hour after phenylalanine administration (11) The loading tests were performed in order to classify children with elevated blood phenylalanine The children were usually loaded at an age of six months

The criteria for HPA were 1) serum phenylalanine below 605 µmol/l (10 mg/100 ml) on a normal dietary intake of phenylalanine (approx 0 7 mmol per kg body weight) 2) a slight rise in serum tyrosine within the first four hours after phenylalanine loading 3) serum phenylalanine returning to preloading levels within 24 hours after a load of 0 6 mmol L phenylalanine per kg body weight (10) The criteria for PKU were 1) serum phenylalanine within 180–425 µmol/l (3–7 mg/100 ml) on a phenylalanine restricted diet (0 09–0 27 mmol per kg body weight per day) 2) no rise in serum tyrosine following phenylalanine loading 3) preloading levels of serum phenylalanine attained 48 hours or more after a load of 0 6 mmol L phenylalanine per kg body weight The criteria for classical versus mild PKU are discussed in a previous paper (11) Briefly to keep serum phenylalanine levels within 180–425 µmol/l (3–7 mg/100 ml) children with classical phenylketonuria tolerate 9–18% and children with the mild form of phenylketonuria 22–36% of a normal daily intake of phenylalanine This distinction cannot be made until the child is more than 2½ years of age (11)

The intellectual development (Cattell) was determined by the same psychologist who was not aware of the diagnoses of the children

RESULTS

Initial blood values

Of 58 newborn infants detected by the Guthrie technique 21 showed an initial blood phenylalanine value of 10 mg/100 ml or lower eleven showed values in between 10 mg/100 ml and 15 mg/100 ml and 26 showed blood phenylalanine values above 15 mg/100 ml (Table 1) Eight of the children in the first group appeared to have PKU and 13 to have HPA In the second group 10 appeared to have PKU and one HPA All the children in the third group appeared to have PKU (Table 1) At the time of screening 23 of the 44 children with PKU were breast fed It should be noticed that only half of the PKU children with Guthrie values below 10 mg/100 ml were breast fed (Fig 1)

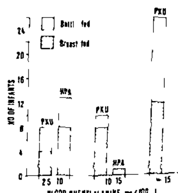


Fig 1 The diet of the neonate at the time of screening related to the Guthrie value in 44 neonates with phenylketonuria (PKU) and 14 neonates with persistent hyperphenylalaninemia (HPA)

Table 2 *Confirmatory test value at days 9 to 22 of life in 55[a] neonates with elevated Guthrie test related to the phenylalanine tolerance at the age of one year*

Diagnosis	Sex	Serum phenylalanine µmol/l (mg/100 ml) <605 (<10)	605–910 (10–15)	>910 (>15)
Serum phenylalanine				
Hyperphenylalaninemia	♂	8	1	0
	♀	5	0	0
Phenylketonuria	♂	0	2	13
	♀		5	19
Phenylalanine tolerance				
Hyperphenylalaninemia				
(mmol/kg/day)		>0.7	>0.7	–
(mg/kg/day)		>116	>116	–
Phenylketonuria				
(mmol/kg/day)[b]		0.18 (0.13–0.19)	0.17 (0.13–0.22)	0.15 (0.12–0.24)
(mg/kg/day)[b]		30 (21–31)	28 (21–36)	25 (20–40)

[a] In three infants dietary treatment was started before the confirmatory test
[b] Median and range in parentheses

Confirmatory serum values

Phenylalanine and tyrosine were determined fluorimetrically in confirmatory tests taken between the 9th and 22nd days of life. The values of phenylalanine and tyrosine as well as the ratio of tyrosine² over phenylalanine and of phenylalanine² over tyrosine differed significantly in infants with PKU as compared to infants with HPA. However, the overlapping ranged from 21–54%. The median serum tyrosine value of infants with PKU was 84 µmol/l (range 41–124 µmol/l) and of infants with HPA 103 µmol/l (range 72–186 µmol/l) $p<0.02$. Thus, no infants with HPA showed

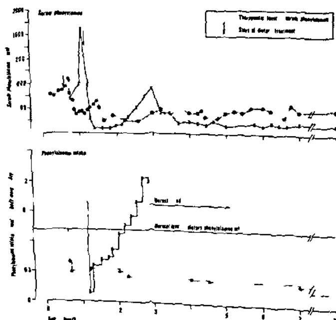


Fig. 2. Course of serum phenylalanine level and dietary phenylalanine tolerance in a case of persistent hyperphenylalaninemia (X) and in a case of phenylketonuria (●).

Table 1 *Blood phenylalanine related to diagnosis in 58 of 488 006 neonates with elevated Guthrie screening test at 5th to 7th days of life*

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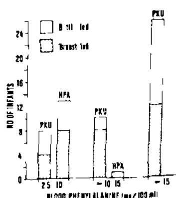


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Phenylketonuria	♂	0	[illegible]	13
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Phenylalanine tolerance				
Hyperphenylalaninemia				
(mmol/kg/day)		>0 7	>0 7	–
(mg/kg/day)		>116	>116	–
Phenylketonuria				
(mmol/kg/day)[b]		0 18 (0 13–0 19)	0 17 (0 13–0 2[illegible])	0 15 (0 1[illegible]–0 [illegible]4)
(mg/kg/day)[b]		30 ([illegible]1–31)	8 ([illegible]1–36)	25 ([illegible]0–40)

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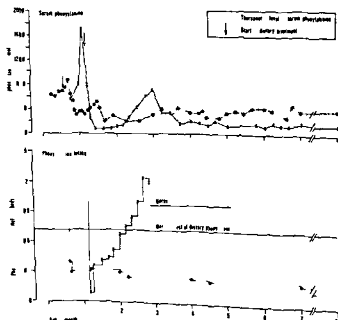


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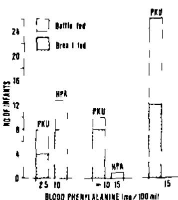


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(mmol/kg/day)		>0 7	>0 7	–
(mg/kg/day)		>116	>116	–
Phenylketonuria				
(mmol/kg/day)[b]		0 18 (0 13–0 19)	0 17 (0 13–0 2_)	0 15 (0 1?–0 ?4)
(mg/kg/day)[b]		30 (21–31)	_8 (?1–36)	25 (?0–40)

[a] In three infants dietary treatment was started before the confirmatory test
[b] Median and range in parentheses

Confirmatory serum values

Phenylalanine and tyrosine were determined fluorimetrically in confirmatory tests taken between the 9th and 22nd days of life The values of phenylalanine and tyrosine as well as the ratio of tyrosine² over phenylalanine and of phenylalanine² over tyrosine differed significantly in infants with PKU as compared to infants with HPA However the overlapping ranged from 21–54% The median serum tyrosine value of infants with PKU was 84 µmol/l (range 41–124 µmol/l) and of infants with HPA 103 µmol/l (range 72–186 µmol/l) $p<0.02$ Thus no infants with HPA showed

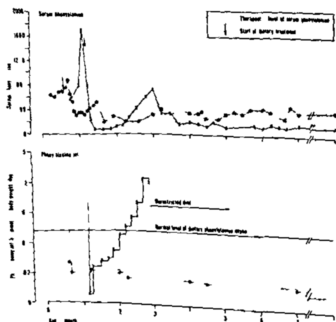


Fig ? Course of serum phenylalanine level and dietary phenylalanine tolerance in a case of persistent hyperphenylalaninemia (X) and in a case of phenylketonuria (●)

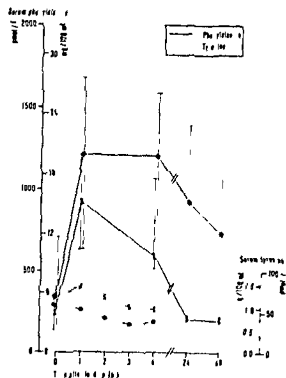


Fig 3 Course of serum phenylalanine and tyrosine after phenylalanine loading of 14 subjects with persistent HPA (X) and 51 subjects with PKU (●) Medians are given and ranges of serum phenylalanine

confirmatory serum tyrosine values below 72 μmol/l (1 3 mg/100 ml)

Nine infants who appeared to have PKU showed confirmatory serum phenylalanine values below 910 μmol/l (15 mg/100 ml) (Table 2) Five of these children have reached an age of 2½ years and it appears that four have the *mild form of PKU and only one the classical* form of PKU Of the 32 children with confirmatory phenylalanine values above 910 μmol/l 22 have reached the age of 2½ years Eighteen of these children have the classical form of PKU and four the mild form of PKU χ^2 7 47 $p<0.01$

Phenylalanine tolerance and load test

Within the first month of life six of 14 children with HPA showed values of serum phenylalanine above 605 μmol/l (10 mg/100 ml) These children were treated according to our practice with a phenylalanine restricted diet However to keep their blood phenylalanine concentration within therapeutic levels the dietary intake of phenylalanine was increased to normal levels within a few months (Fig 2) As shown in Table 2 the phenylalanine tolerance of children with HPA was normal at one year of age

All of the 14 children with HPA showed the same course of *phenylalanine loading* i e no significant increase in serum tyrosine but fasting levels of serum phenylalanine attained within 24 hours after loading (Fig 3) The 24 hour value is significantly different from that observed in children with PKU and no overlapping has hitherto been observed (Fig 3)

DISCUSSION

Regardless of how many forms of disturbances in the metabolism of phenylalanine we may be able to distinguish the possibility to differentiate newborn children who require a dietary therapy from those who do not is essential Recently Bickel (2) and Blaskovics et al (3) *have stressed that the use of an arbitrary* and fixed blood phenylalanine level (e g 20 *mg/100 ml) as the diagnostic criterion for* treatment may be hazardous The present finding that 9 babies who later appeared to have PKU showed confirmatory serum values of phenylalanine (fluorimetric assay) below 910 μmol/l (15 mg/100 ml) between the 9th and 22nd days of life indicates that in these cases *the early clinical diagnosis of PKU can be* made only by weekly estimations of the dietary tolerance of phenylalanine (Fig 2) unless the enzyme activity is determined in liver biopsies

It may be that some of our children classified as PKU would have developed normally on an unrestricted diet *However the* therapeutic level of serum phenylalanine used *in the present study* is generally accepted as beneficial to intellectual development in the treatment of PKU if it is introduced within the first weeks of life (5 13 20) Examination of 72 untreated mentally retarded patients with *PKU revealed 5 patients with phenylalanine* levels (fluorimetric assay) below 910 μmol/l

(15 mg/100 ml) in three separate specimens on a normal protein intake Furthermore six untreated mentally retarded patients with PKU with serum phenylalanine levels below 1031 µmol/l (17 mg/100 ml) and no explanation of their mental deficiency other than PKU showed phenylalanine loading tests consistent with PKU but not with HPA (data to be published)

The finding that 18 of 32 newborns with Guthrie values below 15 mg/100 ml later appeared to have PKU could not be due to the age at testing as all babies were tested between the 5th and 7th days of life nor to the diet of the baby e g breast feeding versus an infant formula (Fig 1) (4 6) However the type of phenylalanine hydroxylase deficiency e g mild versus classical PKU may explain the low Guthrie screening value

The children with HPA reported in this study may be similar to the cases studied by Levy et al (14) None of their subjects had received any form of dietary treatment However the I Q s of the affected subjects were normal and comparable to those of the unaffected siblings School grades of the affected children attending school were good In the present study 6 of 14 children with HPA were given phenylalanine free hydrolysates during their first month of life in low amounts as compared to our schedule for the treatment of PKU The median Cattell scores of the untreated group were 108 (range 107–110) and those of the treated group 108 (range 98–113)

The guide lines for the interpretation of elevated phenylalanine in neonates drawn up in the present study may seem quite simple as compared with the more detailed classification proposed by Berry et al (1) Menkes & Holtzman (15) and Blaskovics et al (3) However it has recently been reported that children with PKU treated within the first 10 days of life obtained higher I Q scores than children started on treatment later (5) The present investigation shows that within the first 10 days of life PKU children may have phenylalanine concentrations between 10 and 15 mg/100 ml We suggest that if the phenylalanine concentration in a confirmatory test is between 605 and 910 µmol/l (10–15 mg/100 ml) treatment with phenylalanine restricted diet should be undertaken The serum phenylalanine concentration should be tested with intervals of 2 days and the phenylalanine intake maintained at a sufficiently high level to keep serum phenylalanine levels in the range of 180–425 µmol/l (3–7 mg/100 ml) A firm diagnosis can be established within the following weeks using estimations of the phenylalanine tolerance of the baby Finally it should be noticed that this article does not deal with transient elevations of both phenylalanine and tyrosine or permanent elevation of phenylalanine due to tyrosinosis Out of 488 006 neonates screened in Denmark we have not detected any case of phenylalanine transaminase deficiency nor of dihydropteridine reductase deficiency (12)

ACKNOWLEDGEMENT

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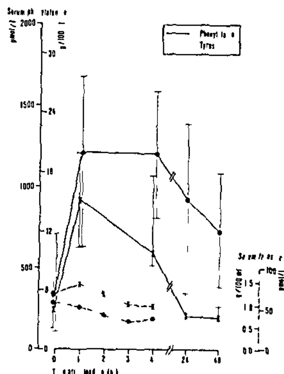


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Submitted July 21 1976
Accepted Oct 21 1976

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Acta Pædiatr Scand 66 345–348 1977

EFFECT OF OBESITY ON THE HYPOTHALAMO PITUITARY-GONADAL FUNCTION IN CHILDHOOD

E CACCIARI A CICOGNANI P PIRAZZOLI F ZAPPULLA P TASSONI F BERNARDI S SALARDI and L MAZZANTI

From the Department of Paediatrics University of Bologna Bologna Italy

ABSTRACT Cacciari E Cicognani A Pirazzoli P Zappulla F Tassoni P Bernardi F Salardi S and Mazzanti L (Department of Paediatrics University of Bologna Bologna Italy) Effect of obesity on the hypothalamo-pituitary-gonadal function in childhood Acta Paediatr Scand 66 345 1977.—In 22 normal and 35 obese boys a gonadal function test (2 000 IU of hCG i m daily for three days and assays of plasma testosterone before and after the hCG administration) was carried out All the short normal children and 31 obese subjects underwent the LH RH test (50 μg i v) While basal testosterone was similar in the two groups of children after hCG testosterone was significantly ($p<0.001$) lower in the obese boys In the normal children a significant positive correlation between bone age and basal and after hCG testosterone was demonstrated this correlation was not found in the obese boys The pituitary reserve of gonadotrophins did not show significant differences between the two groups of children Finally a significant positive correlation ($p<0.01$) between the LH curve area during the LH RH test and bone age was found only in the normal boys.

KEY WORDS Obesity testosterone LH FSH

Gabrilove et al (8) found that Cushing s syndrome in the male is often associated with a decreased endocrine testicular efficiency which then becomes normal after the treatment of the disease Hajjar et al (11) showed that an ACTH excess through the adrenal mediation minimizes in the male the testosterone release of the gonads Since Dunkelman et al (7) Garces et al (9) and Migeon et al (12) state that the function of the pituitary adrenal axis is increased in obesity since Borjeson (2) says that the fertility of the obese adult is impaired and since Amatruda et al (1) report less testosterone release in the obese adult we have studied the endocrine function of the gonads and the pituitary reserve of gonadotrophins in the prepubertal obese child

MATERIALS AND METHODS

22 "short normal" boys (chronological age ranged from 5 to 11 7/12 years mean 8 7/12 bone age ranged from 4 8/12 to 11 7/12 years mean 8 6/12) and 35 obese boys (chronological age ranged from 4 6/12 to 12 3/12 years mean 8 9/12 bone age ranged from 4 6/12 to 12 years mean 9 1/12) were studied Bone age was determined according to the Greulich & Pyle tables (10) In the control group the difference between the chronological and the bone age was never greater than six months In the obese children the difference was never greater than 1 1/12 year In the second group obesity had been existing for at least three years and the mean weight was 57% over the ideal weight (from 41% to 98%) according to the Tonelli & Marinelli tables (16) No child had lost any weight in the year preceding this study All the children examined were in the prepubertal stage of sexual development i e the first stage according to Tanner (15) The X rays of the sella turcica of the same children were normal

All of the children following permission from the parents underwent a gonadal function test all the normal children and 31 obese subjects underwent the LH RH (Luteinizing Hormone Releasing Hormone) test The

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or the FSH pituitary reserve As far as the LH is concerned we found a highly significant positive correlation ($r=+0.610$ $p<0.01$) between the area of the curve and bone age only in the normal children No correlation was found between LH and testosterone both in basal conditions and after stimulus

DISCUSSION

Our results show that in the obese child the testosterone release by the gonads after hCG is significantly lower than in normal children The different behaviour of the gonadal endocrine function in the obese child is confirmed by the lack of correlation (which was found on the contrary in the normal child) between basal or after hCG testosterone and bone age Since the pituitary reserve of LH and FSH is similar in the two groups of subjects the different behaviour of the gonadal endocrine function found in the obese child might be originally attributed to the gonads We must point out that since both groups received the same dose of hCG the obese children were given a smaller amount per kg of gonadotrophins and this may be the reason for the lower testosterone response But we must also mention that since the target tissue (the gonads) was the same in the two groups the weight of the child is probably not very important

Another point to be taken into consideration is the different absorption rate of the drug in the two groups but we feel that the duration of the test limits the possibility of such an event We might ask whether the endocrine defect that we found could refer to a simple delay in the gonadal maturation and therefore would disappear during puberty The lower release of testosterone found in the obese adult by Amatruda et al (1) would tend to support a permanent relationship between obesity and the gonadal endocrine function no matter what the age of the patient may be However this fact is not simple to valuate Tanaka et al (14) when examining male patients after surgery noticed a significant decrease in the urinary excretion of 17 ketosteroids together with a significant increase in the urinary excretion of 17 hydroxy-corticosteroids Gabrilove et al (8) and Hajjar et al (11) showed that the adrenal hypersecretion typical of some pathological situations is accompanied by a decrease in the release of testosterone by the male gonads All these data seem to demonstrate a decrease in the testosterone release when there is an adrenal hypersecretion Being aware of the adrenal hypersecretion in obesity (7 9 12) our data seem to confirm the existence of a relationship between the adrenal and the gonadal function in the obese child

ACKNOWLEDGEMENTS

We thank in particular Dr F Enzmann and Dr M Sesso (Farbwerke Hoechst AG) for the generous supply of synthetic LH RH and Alfa Formaceutici for cooperation in the antisera production The skilful technical assistance of Mr L Zannarini is gratefully acknowledged

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Table 1 *Basal and after hCG testosterone in 22 normal and 35 obese boys*

	Basal testosterone (ng/100 ml)		After hCG testosterone (ng/100 ml)	
	Mean	S E M	Mean	S E M
Normal children	15 40	1 49	114 21	9 91
Obese children	19 95	1 90	67 09	5 99

$p<0\ 001$

LH RH test was performed (before the gonadal function test) at 9 a am after an overnight fast using venous injec tion of 50 μg of synthetic LH RH (Farbwerke Hoechst AG) Venous blood for the evaluation of LH and FSH was collected at times 0 15 30 60 and 90 min The gonadal function test was carried out in the following manner hCG (Human Chorionic Gonadotrophin) was administered at a dosage of 2 000 IU i m every day for three days The dose was administered at 9 a m on the first two days and at 6 a m on the third day Immediately before the beginning and at the end of the test a blood sample was collected to assay plasma testosterone (4)

Plasma testosterone was determined according to the radioimmunoassay method of Collins et al (6) The antiserum we used was obtained from rabbits pretreated with an antitubercular vaccine as we have previously re ported (5) The antiserum was used at a dilution of 1 30000 The method sensitivity was 0 5 ng/100 ml the intra assay coefficient of variation was ±4 2% Serum LH and FSH were evaluated according to the double antibody radioimmunoassay method of Reuter et al (13) using hu man pituitary LH and FSH (the radioimmunological equivalent of LH is 2 150 IU as compared to the 68/40 reference preparation of the National Institute for Medical Research (MRC) Mill Hill London for FSH it is 2 800 IU compared to 68/39 reference preparation of MRC) The results are expressed as mIU/ml MRC reference prepara tion The method sensitivity was 0 5 mIU/ml both for LH and FSH The intra assay coefficient of variation was ±7 4% and ±8 5% for LH and FSH respectively

For the statistical analysis of the results the Student s t test was used and the correlation coefficient r was calcu lated

RESULTS

The mean basal plasma testosterone value (Table 1) did not present any significant differ ences between the two groups of children Af ter the hCG stimulus the plasma testosterone level in the obese children was significantly lower ($p<0\ 001$) than that of the normal children (Table 1) In the normal children there is a significant positive correlation between bone age and basal ($r=+0\ 718$ $p<0\ 001$) or after hCG testosterone ($r=+0\ 472$ $p<0\ 05$) This correlation is not found in the obese children

The mean FSH curve during the LH RH test does not show any significant differences between the two groups (Table 2) The mean LH curve is constantly lower in the obese children and the difference is significant only at the 15th minute ($p<0\ 05$) The pituitary re serve of gonadotrophins was also studied by evaluating the peak the maximum increase and the area of the curve (3) (Table 2) and it did not show any significant differences be tween the two groups of children In both the normal children and the obese there is no cor relation between bone age and the basal values

Table 2 *Mean values ±SEM of serum LH and FSH (mIU/ml) in 22 normal and 31 obese boys submitted to the LH RH test (50 μg iv)*

	Time (min)					Peak	Maximum increase	Area of the curve
	0	15	30	60	90			
Normal children								
LH	1 96±0 17	3 92 ±0 64	4 13±0 65	3 67±0 55	3 15±0 40	4 52±0 66	2 56±0 65	19 50±1 97
FSH	2 14±0 22	3 65±0 34	4 95±0 52	5 36±0 51	5 46±0 73	6 27±0 74	4 13±0 63	28 51±2 87
Obese children								
LH	2 03±0 22	2 64 ±0 26	3 37±0 49	2 82±0 37	2 80±0 32	3 75±0 48	1 78±0 44	17 19±1 96
FSH	2 63±0 29	4 16±0 37	4 65±0 44	5 38±0 40	5 13±0 36	5 95±0 42	3 34±0 37	28 50±2 07

$p<0\ 05$

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DISCUSSION

Our results show that in the obese child the testosterone release by the gonads after hCG is significantly lower than in normal children The different behaviour of the gonadal endocrine function in the obese child is confirmed by the lack of correlation (which was found on the contrary in the normal child) between basal or after hCG testosterone and bone age Since the pituitary reserve of LH and FSH is similar in the two groups of subjects the different behaviour of the gonadal endocrine function found in the obese child might be originally attributed to the gonads We must point out that since both groups received the same dose of hCG the obese children were given a smaller amount per kg of gonadotrophins and this may be the reason for the lower testosterone response But we must also mention that since the target tissue (the gonads) was the same in the two groups the weight of the child is probably not very important

Another point to be taken into consideration is the different absorption rate of the drug in the two groups but we feel that the duration of the test limits the possibility of such an event We might ask whether the endocrine defect that we found could refer to a simple delay in the gonadal maturation and therefore would disappear during puberty The lower release of testosterone found in the obese adult by Amatruda et al (1) would tend to support a permanent relationship between obesity and the gonadal endocrine function no matter what the age of the patient may be However this fact is not simple to valuate Tanaka et al (14) when examining male patients after surgery noticed a significant decrease in the urinary excretion of 17 ketosteroids together with a significant increase in the urinary excretion of 17 hydroxy-corticosteroids Gabrilove et al (8) and Hajjar et al (11) showed that the adrenal hypersecretion typical of some pathological situations is accompanied by a decrease in the release of testosterone by the male gonads All these data seem to demonstrate a decrease in the testosterone release when there is an adrenal hypersecretion Being aware of the adrenal hypersecretion in obesity (7 9 12) our data seem to confirm the existence of a relationship between the adrenal and the gonadal function in the obese child

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Table 1 *Basal and after hCG testosterone ın 22 normal and 35 obese boys*

	Basal testosterone (ng/100 ml)		After hCG testosterone (ng/100 ml)	
	Mean	S E M	Mean	S E M
Normal children	15 40	1 49	114 21	9 91
Obese children	19 95	1 90	67 09	5 99

$p<0\ 001$

LH RH test was performed (before the gonadal function test) at 9 a am after an overnight fast using venous injec tion of 50 μg of synthetic LH RH (Farbwerke Hoechst AG) Venous blood for the evaluation of LH and FSH was collected at times 0 15 30 60 and 90 min The gonadal function test was carried out in the following manner hCG (Human Chorionic Gonadotrophin) was administered at a dosage of 2 000 IU i m every day for three days The dose was administered at 9 a m on the first two days and at 6 a m on the third day Immediately before the beginning and at the end of the test a blood sample was collected to assay plasma testosterone (4)

Plasma testosterone was determined according to the radioimmunoassay method of Collins et al (6) The antiserum we used was obtained from rabbits pretreated with an antitubercular vaccine as we have previously re ported (5) The antiserum was used at a dilution of 1 30 000 The method sensitivity was 0 5 ng/100 ml the intra assay coefficient of variation was ±4 2% Serum LH and FSH were evaluated according to the double antibody radioimmunoassay method of Reuter et al (13) using hu man pituitary LH and FSH (the radioimmunological equivalent of LH is 2 150 IU as compared to the 68/40 reference preparation of the National Institute for Medical Research (MRC) Mill Hill London for FSH it is 2 800 IU compared to 68/39 reference preparation of MRC) The results are expressed as mIU/ml MRC reference prepara tion The method sensitivity was 0 5 mIU/ml both for LH and FSH The intra assay coefficient of variation was ±7 4% and ±8 5% for LH and FSH respectively

For the statistical analysis of the results the Student s t test was used and the correlation coefficient r was calcu lated

RESULTS

The mean basal plasma testosterone value (Table 1) did not present any significant differ ences between the two groups of children Af ter the hCG stimulus the plasma testosterone level in the obese children was significantly lower ($p<0\ 001$) than that of the normal children (Table 1) In the normal children there is a significant positive correlation between bone age and basal ($r=+0\ 718$ $p<0\ 001$) or after hCG testosterone ($r=+0\ 472$, $p<0\ 05$) This correlation is not found in the obese children

The mean FSH curve during the LH RH test does not show any significant differences between the two groups (Table 2) The mean LH curve is constantly lower in the obese children and the difference is significant only at the 15th minute ($p<0\ 05$) The pituitary re serve of gonadotrophins was also studied by evaluating the peak the maximum increase and the area of the curve (3) (Table 2) and it did not show any significant differences be tween the two groups of children In both the normal children and the obese there is no cor relation between bone age and the basal values

Table 2 *Mean values ±SEM of serum LH and FSH (mIU/ml) in 22 normal and 31 obese boys submitted to the LH RH test (50 μg iv)*

	Time (min)					Peak	Maximum increase	Area of the curve
	0	15	30	60	90			
Normal children								
LH	1 96±0 17	3 92 ±0 64	4 13±0 65	3 67±0 55	3 15±0 40	4 52±0 66	2 56±0 65	19 50±1 97
FSH	2 14±0 22	3 65±0 34	4 95±0 52	5 36±0 51	5 46±0 73	6 27±0 74	4 13±0 63	28 51±2 87
Obese children								
LH	2 03±0 22	2 64 ±0 26	3 37±0 49	2 82±0 37	2 80±0 37	3 75±0 48	1 78±0 44	17 19±1 96
FSH	2 63±0 29	4 16±0 37	4 65±0 44	5 38±0 40	5 13±0 36	5 95±0 42	3 34±0 37	28 50±2 07

$p<0\ 05$

or the FSH pituitary reserve As far as the LH is concerned we found a highly significant positive correlation ($r = +0.610$ $p < 0.01$) between the area of the curve and bone age only in the normal children No correlation was found between LH and testosterone both in basal conditions and after stimulus

DISCUSSION

Our results show that in the obese child the testosterone release by the gonads after hCG is significantly lower than in normal children The different behaviour of the gonadal endocrine function in the obese child is confirmed by the lack of correlation (which was found on the contrary in the normal child) between basal or after hCG testosterone and bone age Since the pituitary reserve of LH and FSH is similar in the two groups of subjects the different behaviour of the gonadal endocrine function found in the obese child might be originally attributed to the gonads We must point out that since both groups received the same dose of hCG the obese children were given a smaller amount per kg of gonadotrophins and this may be the reason for the lower testosterone response But we must also mention that since the target tissue (the gonads) was the same in the two groups the weight of the child is probably not very important

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Submitted Aug 11 1976
Accepted Oct 29 1976

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Acta Pædiatr Scand 66 349–354 1977

ASYMPTOMATIC BACTERIURIA IN SCHOOLGIRLS

VI The Correlation between Urinary and Faecal Escherichia coli Relation to the Duration of the Bacteriuria and the Sampling Technique

G LIDIN JANSON and U LINDBERG

From the Departments of Clinical Bacteriology and Immunology Institute of Medical Microbiology and Departments of Infectious Diseases and Pediatrics University of Goteborg Goteborg Sweden

ABSTRACT Lidin Janson G and Lindberg U (Departments of Clinical Bacteriology and Immunology Institute of Medical Microbiology and Departments of Infectious Diseases and Pediatrics University of Goteborg Goteborg Sweden) Asymptomatic bacteriuria in school girls VI The correlation between urinary and faecal *Escherichia coli* Relation to the duration of the bacteriuria and the sampling technique Acta Paediatr Scand 66 349 1977.—The occurrence of the urinary strain in the anus rectum and faeces was investigated in 27 girls with asymptomatic bacteriuria (ABU) In patients with bacteriuria of relatively short duration 46 % of the faecal isolates were of the urinary strain as compared to only 18 % in patients with bacteriuria of relatively long duration In general the correlation between the urinary and faecal flora is striking at the time of establishment of ABU but diminishes with time The diminished correlation may be due to two factors firstly the composition of the faecal flora changes with time Secondly the correlation may be obscured by complex changes in the properties of bacterial strains established in the urinary tract Contamination by the infected urine did not seem to be a serious problem when the rectal mucosa was swabbed proximal to the anal canal

KEY WORDS Asymptomatic bacteriuria *E coli* faecal flora

Escherichia coli infecting the urinary tract usually originate in the patient s own bowel flora (17) Analyses of the correlation between the urinary and the faecal flora may be of importance for an understanding of the host parasite relationship in urinary tract infection (15) Such analyses have to take into account the two possibilities of contamination of the faecal specimen by the infected urine and of colonization of the gut by a strain already established in the urinary tract (3 7) A longitudinal study of schoolgirls with asymptomatic bacteriuria (9) offered an opportunity to study the problems of sampling the faecal flora in bacteriuria and to analyse the relationship between the urinary and faecal flora

MATERIAL AND METHODS

Patients

The patients were all included among the schoolgirls with asymptomatic bacteriuria (ABU) described and prospectively followed up treated or untreated by Lindberg (9 10) Twenty seven bacteriuric children attending the clinic for their scheduled appointments during a five month period took part in this study Patients harbouring O typable urinary strains were preferred The 27 patients were grouped according to the duration of their ABU Seventeen patients were classified as having bacteriuria of relatively long duration They were either still bacteriuric with the strain originally found on screening or had a re infection strain carried for at least one year Two of these patients had new infections during the study period Together with ten others they were then classified as having ABU of relatively short duration that is reinfections of 0–11 months duration

Sampling

Without preliminary cleansing a cottonwool swab was rotated superficially in the anus After that two swab

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Table 3 *Comparison between urinary and faecal E coli on two or three occasions in nine episodes of asymptomatic bacteriuria*

Girl	Duration of bacteriuria with present strain months	Urinary strain	Number of colonies from each site corresponding to urinary strain					
			Anus	Rectum tube not used	Rectum tube used	Faeces swab no I	Faeces swab no II	Faeces swab no III
ME	0– 2	O75 KNtH+	9	9	8	0/2[a]	4/6	–[c]
ME	2– 4	O25 KNtH+	10	10	6	1		5
EL	0– 4	O7 K1	10	10	10	10	8	10
EL	4– 8	O7 K1	0	0	0	0	0	0
MH	0– 6	O6 KNtH+	10	10	10	10	8	10
MH	6–11	O6 KNtH+	0	0	0	0	0	0
EA	12–18	O1 K51	0	3	0	0	0	2
EA	13–19	O1 K51	0	0	0	7	8	6
TS	13–18	O1 K1	0	0	0	0	0	0
TS	15– 0	O1 K1	0	0	0	–	0	–
KK	> 8	O16 (Sa)K1–H+	9	10	10	9	10	10
KK	>10	O16 (Sa)K1–H+	4	2	8	0	0	0
AKW	> 8	O17 KNt	9	7	8	8	10	10
AKW	> 9	O17 KNt	8	3	5	3	6	4
AKW	>11	O17 KNt	3	0	0	0	0	0
MJ	>11	O75 KNtH+	1	0+[f]	0+	0+	0+	0+
MJ	>12	O75 KNtH+	0	0	0	0	0	0
AS	>19	O75 KNt	1	2	2	0	1	0
AS	>21	O75 KNt	0/3	8	4	0+	2	–

H+=haemolysis in solid medium

[a] Only two out of ten cultures grew enterobacteria Neither of two colonies corresponded to the urinary strain

Only six out of ten cultures grew enterobacteria Four out of six colonies corresponded to the urinary strain

[c] None out of ten cultures grew enterobacteria

After a ten-day course of nitrofurantoin one negative urine culture obtained in EL 4 months and in EA 3 weeks before indicated specimen

[f] 0+=very small amount of strain found by selection for colony morphology

covery of the urinary strains from the swabs from the anus and rectum is summarized in Table 1 In no instance was the urinary strain recovered from the rectum but not from the anus In eight instances it was found in the anus but not in the plain rectal swab and in seven cases in the anus but not in the rectal swab taken through the tube In these specimens the urinary strain thus occurred more often in the anal swab than in either of the rectal swabs (Sign test)

The overall proportion of stool isolates conforming with the urinary strain present was 399/875 isolates (45 6%) in the group with bacteriuria of short duration and 250/1404 (17 8%) in bacteriuria of long duration The proportions of isolates corresponding to the urinary strain found among 60 randomly selected colonies were compared for the twelve episodes of bacteriuria of short duration (*A*) and the seventeen episodes of long duration (*B*) Table 2 In group *A* 5 patients had 0–20 concordant colonies and 6 had 41–60 colonies In group *B* 15 girls had only 0–20 colonies conforming to the urinary strain The two distributions are different at the 5% level of significance (Fisher s permutation test)

The same bacteriuria strain was found more than once in nine girls The characteristics of the urinary strains and the numbers of colonies from each sampling site—anus rectum and faeces—that corresponded to the urinary strain of each patient are shown in Table 3

Three girls (ME EL and MH) had recent re

Table 1 *Occurrence in swabs from the anus (A) and the rectum (R) of twenty two urinary strains not recovered among thirty randomly selected colonies from the innermost part of the faeces*

One rectal swab (R_2) was taken through a sterile tube inserted into the anal canal

Pattern of occurrence of the urinary strain	No of cases
$A-R_1-R_2-$	11
$A+R_1+R_2+$	3
$A+R_1-R_2-$	7
$A+R_1-R_2+$	1
$A-R_1$ and/or R_2+	0

specimens were taken from the rectum the stick inserted to a mark 40 mm from the tip In endeavour to minimize contamination from the anal canal one of the swabs was inserted through a small tube The tubes were made from heat sterilizable nylon They were 30 mm long with an internal diameter of 8 mm a rounded inner tip and a flange at the outer end Sterile vaseline was applied to the tip and the tube was passed through the anal canal the flange being firmly pressed against the skin The order of taking the two rectal swabs in each case was randomized

Each swab was put into a tube with transport medium (13) and kept at +4 C until cultured usually later the same day Material for the collection of faecal specimens at home was dispensed and careful instructions were given The girl was asked first to urinate into the lavatory basin After wiping herself carefully she was then to deliver the faeces directly into a large plastic bag placed in a suitable container The bag was to be sealed and put into an insulating box with cold accumulators The boxes were collected from the patients homes usually within twelve hours of defaecation

Bacteriological procedure

The faecal specimens which were all formed were cut into pieces using a red hot metal rod At each of three roughly equidistant points one swab was bored into the centre of one cut surface There were thus six swabs from each patient one anal two rectal and three faecal swabs

Each swab was inoculated ten times on a modified Conradi Drigalski agar The inocula were spread with a platinum loop The plates were incubated aerobically at 37 C overnight

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Statistical calculations

The sign test (4) and Fisher s permutation test (14) were used

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In twenty two episodes of bacteriuria the urinary strain was not found on at least one occasion among thirty randomly selected isolates from the innermost part of the faeces The re

Table 2 *Proportion of faecal isolates conforming to the urinary strain in twelve episodes of bacteriuria of short duration (A) and in seventeen episodes of long duration (B)*

Two girls had two episodes each

Number of concordant colonies among 60 randomly selected colonies	Bacteriuria group	
	A	B
0–20	5	15
21–40	1	
41–60	6	2

Table 3 *Comparison between urinary and faecal E coli on two or three occasions in nine episodes of asymptomatic bacteriuria*

Girl	Duration of bacteriuria with present strain months	Urinary strain	Number of colonies from each site corresponding to urinary strain					
			Anus	Rectum tube not used	Rectum tube used	Faeces swab no I	II	III
ME	0– 2	O75 KNtH+	9	9	8	0/2[a]	4/6	–[d]
ME	2– 4	O25 KNtH+	10	10	6	1	2	5
EL	0– 4	O7 K1	10	10	10	10	8	10
EL	4– 8	O7 K1	0	0	0	0	0	0
MH	0– 6	O6 KNtH+	10	10	10	10	8	10
MH	6–11	O6 KNtH+	0	0	0	0	0	0
EA	12–18	O1 K51	0	3	0	0	0	2
EA	13–19[e]	O1 K51	0	0	0	7	8	6
TS	13–18	O1 K1	0	0	0	0	0	0
TS	15–20	O1 K1	0	0	0	–	0	–
KK	> 8	O16 (Sa)K1–H+	9	10	10	9	10	10
KK	>10	O16 (Sa)K1–H+	4	[illegible]	8	0	0	0
AKW	> 8	O17 KNt	9	7	8	8	10	10
AKW	> 9	O17 KNt	8	3	5	3	6	4
AKW	>11	O17 KNt	3	0	0	0	0	0
MJ	>11	O75 KNtH+	1	0+[f]	0+	0+	0+	0+
MJ	>12	O75 KNtH+	0	0	0	0	0	0
AS	>19	O75 KNt	1	[illegible]	2	0	1	0
AS	>21	O75 KNt	0/3	8	4	0+	[illegible]	2

H+=haemolysis in solid medium
[a] Only two out of ten cultures grew enterobacteria Neither of two colonies corresponded to the urinary strain
Only six out of ten cultures grew enterobacteria Four out of six colonies corresponded to the urinary strain
[d] None out of ten cultures grew enterobacteria
After a ten-day course of nitrofurantoin one negative urine culture obtained in EL 4 months and in EA 3 weeks before indicated specimen
[f] 0+=very small amount of strain found by selection for colony morphology

covery of the urinary strains from the swabs from the anus and rectum is summarized in Table 1 In no instance was the urinary strain recovered from the rectum but not from the anus In eight instances it was found in the anus but not in the plain rectal swab and in seven cases in the anus but not in the rectal swab taken through the tube In these specimens the urinary strain thus occurred more often in the anal swab than in either of the rectal swabs (Sign test)

The overall proportion of stool isolates conforming with the urinary strain present was 399/875 isolates (45 6%) in the group with bacteriuria of short duration and 250/1 404 (17 8%) in bacteriuria of long duration The proportions of isolates corresponding to the urinary strain found among 60 randomly selected colonies were compared for the twelve episodes of bacteriuria of short duration (*A*) and the seventeen episodes of long duration (*B*) Table 2 In group *A* 5 patients had 0–20 concordant colonies and 6 had 41–60 colonies In group *B* 15 girls had only 0–20 colonies conforming to the urinary strain The two distributions are different at the 5% level of significance (Fisher s permutation test)

The same bacteriuria strain was found more than once in nine girls The characteristics of the urinary strains and the numbers of colonies from each sampling site—anus rectum and faeces—that corresponded to the urinary strain of each patient are shown in Table 3

Three girls (ME EL and MH) had recent re

Table 1 *Occurrence in swabs from the anus (A) and the rectum (R) of twenty two urinary strains not recovered among thirty randomly selected colonies from the innermost part of the faeces*

One rectal swab (R_2) was taken through a sterile tube inserted into the anal canal

Pattern of occurrence of the urinary strain	No of cases
$A-R_1-R_2-$	11
$A+R_1+R_2+$	3
$A+R_1-R_2-$	7
$A+R_1-R_2+$	1
$A-R_1$ and/or R_2+	0

specimens were taken from the rectum the stick inserted to a mark 40 mm from the tip In endeavour to minimize contamination from the anal canal one of the swabs was inserted through a small tube The tubes were made from heat sterilizable nylon They were 30 mm long with an internal diameter of 8 mm a rounded inner tip and a flange at the outer end Sterile vaseline was applied to the tip and the tube was passed through the anal canal the flange being firmly pressed against the skin The order of taking the two rectal swabs in each case was randomized

Each swab was put into a tube with transport medium (13) and kept at +4 C until cultured usually later the same day Material for the collection of faecal specimens at home was dispensed and careful instructions were given The girl was asked first to urinate into the lavatory basin After wiping herself carefully she was then to deliver the faeces directly into a large plastic bag placed in a suitable container The bag was to be sealed and put into an insulating box with cold accumulators The boxes were collected from the patients homes usually within twelve hours of defaecation

Bacteriological procedure

The faecal specimens which were all formed were cut into pieces using a red hot metal rod At each of three roughly equidistant points one swab was bored into the centre of one cut surface There were thus six swabs from each patient one anal two rectal and three faecal swabs

Each swab was inoculated ten times on a modified Conradi Drigalski agar The inocula were spread with a platinum loop The plates were incubated aerobically at 37 C overnight

The last colony growing from each of the ten cultures was then subcultured as were representatives of any colony types not included among these The subcultures were typed as previously described (8) The procedure included a simplified *E coli* O grouping using mono and multivalent antisera against 69 O groups in all Strains that agglutinated spontaneously were designated Sa Haemolysin production was assessed in plates with 5% washed horse erythrocytes Antibiotic sensitivity was tested using a disc diffusion method (5) Strains conforming to the urinary strain with respect to O agglutination pattern haemolytic capacity and antibiogram were preserved as deep agar stab cultures under sterile paraffin oil usually one colony from each positive site Urinary strains were preserved in the same manner The preserved strains were later subjected to a limited K antigen typing with the serum agar technique (2) Strains giving no precipitate around the colony on any of the plates were designated KNt O and K nontypable strains found in the urine and the faeces of the same patient were biotyped according to their pattern of fermentation of 16 carbohydrates as described by Bettelheim & Taylor (1)

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Thirty nine sets of specimens were obtained from the 27 patients All but two of the anal swabs and five of the swabs from two faecal specimens yielded *Enterobacteriaceae* from all ten spread out cultures Altogether 2 279 colonies were randomly selected and typed

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0–20	5	15
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Table 3 *Comparison between urinary and faecal E coli on two or three occasions in nine episodes of asymptomatic bacteriuria*

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AKW	>11	O17 KNt	3	0	0	0	0	0
MJ	>11	O25 KNtH+	1	0+§	0+	0+	0+	0+
MJ	>12	O25 KNtH+	0	0	0	0	0	0
AS	>19	O75 KNt	1	2	2	0	1	0
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* Only two out of ten cultures grew enterobacteria Neither of two colonies corresponded to the urinary strain

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duration was defined as a fairly recent recurrence established within 0–11 months Bacteriuria of long duration was defined as either persistence of the strain harboured when bacteriuria was first detected at screening or of a recurrence established at least one year earlier

In the first group 46% of the pooled faecal isolates corresponded to the urinary strain compared to 18% in the second group In the individual patients the proportions of the urinary strains found among 60 faecal isolates were differently distributed in the short-duration and the long-duration bacteriuria group (*Table 2*) In the former group there was a bimodal distribution and in the latter there was a shift towards a low proportion of the urinary strain in the faecal flora The development was clearly illustrated by some of the girls in whom the same bacteriuria strain was found more than once (Table 3)

These findings corroborate the interpretation given by Roberts et al (15) to their results from a study of bacteriuria in non pregnant women The correspondence between urinary and faecal *E coli* was lower in ABU than in symptomatic infection Since bacteria infecting the urinary tract commonly originate in the patient s faecal coliform flora (7 17 18) and since the faecal flora changes at intervals (16) it was concluded that when symptoms develop they probably do so in most cases within a short time of the infection becoming established In most cases of ABU on the other hand the infection will have been established long enough for the faecal flora to have changed (15) The possibility of colonization of the gut from the urinary tract (3 15 18) might explain the divergent findings in the rectal swabs and the faeces proper in a few cases e g EA and KK Table 3 It may well be that urine containing at times millions of viable *E coli* per millilitre exerts a stabilizing influence on the normal flora of either mainly the rectum *via* true retrograde colonization or also of the faeces proper *via* the retrograde or the oral route Such a mechanism might explain some recurrences with strains identical to that found at the preceding infection as in the girls EA and EL (Table 3)

Generally however the dynamics of the faecal flora will in time eliminate the strain that once became established in the urinary tract In the urinary tract on the other hand the strain may undergo complex changes of its antigenic and biochemical properties (1 11) In one girl (EJ) the present urinary *E coli* SaK1 was considered to be a degraded variant of the O18 K1 originally found in her urine and still a minority strain in her faecal flora Thus a strain may sometimes remain stable in the faecal flora while the clone established in the urinary tract changes under selective pressures exerted in this milieu

ACKNOWLEDGEMENTS

This work was supported by grants from the Medical Faculty of Göteborg the Göteborg Medical Association the First of May Flower Annual Campaign for Children s Health and the Swedish Medical Research Council (Project no 215)

The skilful technical assistance of Helena Lomberg Ingela Delgado Lilian Nilsson and Stina Wallenkrans is very much appreciated

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currences of asymptomatic bacteriuria with a strain not present at the latest check up two to six months earlier In all of these there was a strong correlation between urinary and faecal *E coli* At the next control examination, two to five months later the correlation had disappeared in two of the girls, EL and MH A similar pattern was observed in KK and AKW both of whom had entered the ABU study eight months earlier when their bacteriuria was detected at screening In KK on the second occasion the strain was still found in the anal and rectal swabs, but not in the faeces proper In one girl (MJ), only selection for colony morphology showed the urinary strain to be present in the rectum and faeces

On the first sampling occasion three girls (EA TS and AS) harboured a urinary strain which had been present for at least one year In TS, the strain was not found in any of the swabs and in AS it occurred in varying proportions at all sites In EA three colonies conforming to the urinary strain were found in the rectum and two in the faeces proper on the first occasion On the second occasion more than half of the colonies from the innermost part of the faeces but none of the isolates from the anus or rectum were of the same strain In this girl as in EL one negative urine culture had been obtained after a short course of nitrofurantoin, but bacteriuria with the same strain had recurred

One girl EJ not included in Table 3 had originally harboured a urinary *E coli* O 18 K1 biotype A (1) At the time of the study the urinary strain was SaK1 not fermenting salicin but otherwise with the same fermentation pattern In one rectal and one faecal swab one isolate indistinguishable from the original bacteriuria strain was found

DISCUSSION

One of the aims of the present study was to study the methodological problems involved in sampling the faecal flora in bacteriuria In a preliminary study (unpublished) 33 girls with asymptomatic bacteriuria had their anuses swabbed before and on the morning after a bladder wash out with neomycin solution (6 12) In 20 cases a negative urinary culture was obtained at the same time as the second anal swab A strain corresponding to the urinary *E coli* was found in 12 girls both before and after the wash out in 13 patients neither before nor after, and in 8 before but not after the wash out In these cases the recovery probably represented passive contamination of the anal region and not true colonization

In endeavour to obtain a clean catch faecal specimen one approach was to analyze the interior of a specimen of faeces delivered as far as possible separately from urination This should represent the gut flora oral to the rectum

Another approach was to sample the flora of the rectal ampulla The swab then had to pass through the probably contaminated anus We tried to assess the importance of this by analyzing duplicate rectal swabs one being introduced through a small tube The tip of the tube may have carried some urinary bacteria so the shielding was not expected to be perfect The variation between the results of the two rectal swabs in each patient was however strikingly small and very similar to that between different swabs from the same specimen of faeces proper When the urinary strain corresponded to less than one in thirty colonies from the innermost part of the faeces correspondence was greater in the anal swabs than in the rectal swabs of either kind (Table 1) Thus when the rectal mucosa was swabbed proximal to the anal canal superficial contamination did not seem to be of any great importance

The apparent degree of correlation between urinary and faecal flora in patients with bacteriuria may thus be influenced by the sampling technique Apart from this an important difference was revealed when the girls of the present study were divided into two groups depending on the duration of their bacteriuria with the present strain Bacteriuria of short

Acta Pædiatr Scand 66 355–360 1977

CORD SERUM LIPID AND LIPOPROTEIN CHOLESTEROL VALUES IN NORMAL AND BETAMETHASONE TREATED NEWBORNS OF VARYING GESTATIONAL AGE

G E ANDERSEN and B FRIIS HANSEN

From the Neonatal Department Rigshospitalet Copenhagen Denmark

ABSTRACT Andersen G E and Friis-Hansen B (Neonatal Department Rigshospitalet Copenhagen Denmark) Cord serum lipid and lipoprotein-cholesterol values in normal and betamethasone-treated newborns of varying gestational age Acta Paediatr Scand 66 355 1977 —Cord serum total cholesterol very low density lipoprotein low density lipoprotein high density lipoprotein-cholesterol and triglyceride was determined in 30 AGA infants and 35 SGA infants born between the ≥37th≤42th week of gestation and in 26 AGA infants born between the ≥33th<37th week of gestation In SGA infants significantly higher VLDL-cholesterol and triglyceride values were found than in AGA infants In AGA infants <37 weeks of gestation total cholesterol LDL- and HDL-cholesterol concentrations were higher than in AGA infants ≥37 weeks of gestation In 10 betamethasone treated AGA infants <37 weeks of gestation HDL-cholesterol was higher than in 16 untreated AGA infants <37 weeks of gestation

KEY WORDS cord serum prematures small for dates cholesterol triglyceride low density lipoproteins high density lipoproteins betamethasone

Since 1960 several papers have been published dealing with lipids and lipoproteins in normal newborns However very little has been published so far about lipid and lipoprotein values in newborns with low gestational age or with abnormal lipid metabolism In 1973 Kwiterovich et al (1) demonstrated that dominantly inherited familial hypercholesterolemia (FH) can be diagnosed at birth by estimating the concentration of cord serum low density lipoprotein (LDL) cholesterol in children having one parent with the disease Recently Andersen & Friis Hansen (2) have shown this to be the case also for newborns with unknown parental phenotypes Further more Tsang et al (3) as well as Andersen & Friis Hansen (4) have shown that transient neonatal hypertriglyceridemia (TNH) may be a sign of intrauterine growth retardation (IUGR) and/or perinatal asphyxia All these studies have been carried out in mature newborns We here present data of lipid and lipoprotein-cholesterol values in cord blood from full term and prematurely delivered newborns including AGA (appropriate for gestational age) and SGA (small for gestational age) infants Furthermore the preliminary results of the influence of betamethasone on cord serum lipids and lipoprotein-cholesterol in prematurely delivered newborns are presented

The study shows that it is of major importance to evaluate the gestational age of the newborn infant to avoid false diagnoses of FH and TNH since in SGA infants cord serum triglyceride values and in AGA infants <37

Abbreviations VLDL very low density lipoprotein LDL low density lipoprotein HDL high density lipoprotein TNH transient neonatal hypertriglyceridemia FH familial hypercholesterolemia IUGR intrauterine growth retardation

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Submitted Aug 12 1976
Accepted Nov 16 1976

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Acta Pædiatr Scand 66 355-360 1977

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ABSTRACT Andersen G E and Friis Hansen B (Neonatal Department Rigshospitalet Copenhagen Denmark) Cord serum lipid and lipoprotein-cholesterol values in normal and betamethasone treated newborns of varying gestational age Acta Paediatr Scand 66 355 1977 —Cord serum total cholesterol very low density lipoprotein low density lipoprotein high density lipoprotein-cholesterol and triglyceride was determined in 30 AGA infants and 35 SGA infants born between the ≥37th≤42th week of gestation and in 26 AGA infants born between the ≥33th<37th week of gestation In SGA infants significantly higher VLDL-cholesterol and triglyceride values were found than in AGA infants In AGA infants <37 weeks of gestation total cholesterol LDL- and HDL-cholesterol concentrations were higher than in AGA infants ≥37 weeks of gestation In 10 betamethasone treated AGA infants <37 weeks of gestation HDL-cholesterol was higher than in 16 untreated AGA infants <37 weeks of gestation

KEY WORDS cord serum prematures small for dates cholesterol triglyceride low density lipoproteins high density lipoproteins betamethasone

Since 1960 several papers have been published dealing with lipids and lipoproteins in normal newborns However very little has been published so far about lipid and lipoprotein values in newborns with low gestational age or with abnormal lipid metabolism In 1973 Kwiterovich et al (1) demonstrated that dominantly inherited familial hypercholesterolemia (FH) can be diagnosed at birth by estimating the concentration of cord serum low density lipoprotein (LDL) cholesterol in children having one parent with the disease Recently Andersen & Friis Hansen (2) have shown this to be the case also for newborns with unknown parental phenotypes Furthermore Tsang et al (3) as well as Andersen & Friis Hansen (4) have shown that transient neonatal hypertriglyceridemia (TNH) may be a sign of intrauterine growth retardation (IUGR) and/or perinatal asphyxia All these studies have been carried out in mature newborns We here present data of lipid and lipoprotein-cholesterol values in cord blood from full term and prematurely delivered newborns including AGA (appropriate for gestational age) and SGA (small for gestational age) infants Furthermore the preliminary results of the influence of betamethasone on cord serum lipids and lipoprotein-cholesterol in prematurely delivered newborns are presented

The study shows that it is of major importance to evaluate the gestational age of the newborn infant to avoid false diagnoses of FH and TNH since in SGA infants cord serum triglyceride values and in AGA infants <37

Abbreviations VLDL very low density lipoprotein LDL low density lipoprotein HDL high density lipoprotein TNH transient neonatal hypertriglyceridemia FH familial hypercholesterolemia IUGR intrauterine growth retardation

Table 1 *Cord serum lipid and lipoprotein cholesterol concentrations in mmol/l in 30 mature newborns*

AGA infants born between the ≥37th≤42th week of gestation

Birth wt (g)	Gest age	Sex	T C	VLDL C	LDL-C	HDL-C	TG
3 120	39	F	1 47	0 21	0 42	0 84	0 46
3 480	39	M	1 83	0 24	0 50	1 09	0 52
3 745	39	M	2 14	0 21	0 79	1 14	0 46
3 800	39	M	2 07	0 23	0.52	1 32	0 49
3 050	40	M	1 34	0 15	0 37	0 82	0 32
3 090	40	F	1 75	0 30	0 59	0 86	0 64
3 100	40	F	1 37	0 18	0 39	0 80	0 39
3 150	40	F	1 69	0 20	0 58	0 90	0 42
3 170	40	M	1 66	0 15	0 60	0 91	0 33
3 250	40	F	1 68	0 33	0 55	0 80	0 72
3 400	40	F	1 74	0 23	0 53	0 98	0 49
3 425	40	F	1 49	0 17	0 49	0 83	0 37
3 530	40	F	1 89	0 11	0 43	1 35	0 23
3 550	40	M	1 76	0 20	0 55	1 01	0 42
3 560	40	F	1 41	0 16	0 27	0 98	0 34
3 600	40	M	1 93	0 27	0 74	0 92	0 59
3 680	40	F	1 67	0 31	0 46	0 90	0 67
3 700	40	M	1 85	0 13	0 71	1 01	0 29
3 700	40	F	1 79	0 28	0 54	0 97	0 61
3 710	40	M	2 04	0 25	0 74	1 05	0 54
3 750	40	M	1 66	0 24	0 62	0 80	0 52
3 750	40	M	1 75	0 19	0 49	1 07	0 40
3 800	40	M	1 97	0 26	0 74	0 97	0 55
3 950	40	M	1 37	0 16	0 47	0 74	0 34
4 485	40	F	1 79	0 12	0 55	1 12	0 26
3 420	41	M	1 39	0 17	0 43	0 79	0 37
3 450	41	F	2 27	0 18	0 72	1 37	0 38
3 600	4	F	1 54	0 12	0 40	1 02	0 26
3 760	41	F	1 90	0 16	0 78	0 96	0 35
3 900	41	F	1 87	0 21	0 38	1 28	0 46
10th percentile							
3 102	39		1 37	0 12	0 38	0 80	0 26
Median							
3 580	40		1 75	0 20	0 53	0 97	0 42
90th percentile							
3 890	41		2 07	0 30	0 74	1 32	0 64

weeks of gestation cord serum LDL cholesterol values are found to be much higher than in full term mature newborns

In betamethasone treated AGA infants born before the 37th week of gestation the concentration of cord serum HDL cholesterol was found to be higher than in untreated AGA infants below 37 weeks of gestation

MATERIAL

All children in the study were taken from our screening program for hyperlipoproteinemia which so far includes 7065 consecutive live born infants from Copenhagen Only newborns without signs of perinatal asphyxia (i e no meconium stained amniotic fluid fetal heart rate above 120/min during delivery no circumflexion of the umbilical cord and 1 min AS>7) were chosen to avoid cases with TNH Otherwise no selection of the newborns was made If the birthweight was above the 10th percentile value for gestation the infant was classified as AGA If the birth weight was below the 10th percentile value for gestation the infant was classified as SGA

A total of 30 full term mature newborns 35 full term SGA infants and 26 purely premature newborns were studied 10 of whom had been treated with betamethasone prior to delivery Another 5 newborns being both *SGA and prematurely born were not included in the* study since this number was too small for statistical evaluation

The betamethasone treatment was given in the following way

Table 2 *Cord serum lipid and lipoprotein cholesterol concentrations in mmol/l in 35 SGA infants born between the ≥37th≤42th week of gestation*

Birth wt (g)	Gest age	Sex	T-C	VLDL-C	LDL-C	HDL-C	TG
1 850	37	F	1.84	0.35	0.76	0.73	0.75
1 950	37	F	1.96	0.41	0.68	0.87	0.89
1 950	37	M	2.21	0.7[illegible]	0.50	0.99	1.55
2 300	38	M	1.54	0.51	0.49	0.54	1.10
2 350	38	F	1.44	0.6[illegible]	0.11	0.71	1.33
2 450	38	M	1.47	0.38	0.59	0.50	0.8[illegible]
2 490	38	M	1.84	0.[illegible]8	0.79	0.77	0.60
2 600	38	F	1.71	0.35	0.67	0.69	0.76
2 300	39	M	1.40	0.43	0.37	0.60	0.9[illegible]
[illegible]400	39	M	1.5[illegible]	0.43	0.46	0.63	0.9[illegible]
2 400	39	F	1.64	0.59	0.44	0.61	1.[illegible]7
2 440	39	F	2.09	0.40	0.65	1.04	0.87
2 470	39	M	1.67	0.[illegible]	0.65	0.80	0.48
2 575	39	F	2.16	0.35	0.66	1.15	0.76
2 600	39	M	1.85	0.40	0.59	0.86	0.85
2 600	39	M	1.36	0.60	0.37	0.39	1.30
2 610	39	M	2.30	0.28	0.76	1.[illegible]6	0.61
2 650	39	M	1.[illegible]0	0.33	0.40	0.47	0.71
2 700	39	F	1.66	0.39	0.54	0.73	0.83
1 730	40	F	1.4[illegible]	0.32	0.48	0.62	0.68
2 450	40	M	1.37	0.55	0.25	0.57	1.18
2 500	40	F	2.11	0.38	0.73	1.00	0.8[illegible]
2 500	40	F	2.47	0.31	1.03	1.13	0.66
2 550	40	F	2.23	0.33	0.93	0.97	0.71
[illegible]650	40	F	1.84	0.40	0.5[illegible]	0.92	0.85
2 700	40	F	2.10	0.2[illegible]	0.88	1.00	0.48
2 700	40	F	1.94	0.[illegible]0	0.57	1.17	0.44
2 750	40	M	1.91	0.30	0.65	0.96	0.65
2 750	40	M	1.60	0.45	0.59	0.56	0.96
2 800	40	F	2.[illegible]	0.66	0.81	0.75	1.4[illegible]
2 800	40	M	1.60	0.6[illegible]	0.25	0.73	1.34
2 800	40	F	2.[illegible]0	0.40	0.79	1.01	0.85
2 750	41	F	2.16	0.76	0.67	0.73	1.63
2 600	42	M	1.86	0.37	0.46	1.03	0.80
2 700	4[illegible]	F	2.[illegible]0	0.31	0.75	1.14	0.67
10th percentile							
1 950	38		1.38	0.26	0.3[illegible]	0.5[illegible]	0.55
Median							
2 575	39		1.84	0.39	0.59	0.77	0.83
90th percentile							
2 770	40		2.[illegible]2	0.64	0.84	1.14	1.37

Pregnant mothers entering the Rigshospital in Copenhagen in labor before the 37th week of pregnancy were given 12 mg of betamethasone i m immediately and this was repeated once 24 hours later. In addition 100 mg of Ritodrine in 1000 ml of 5.5% glucose was given i v over a 24 hour period and 100 mg of peroral phenobarbital divided into 3 doses was given daily until birth had taken place.

In the present study no matching was done between pairs of treated and untreated AGA infants <37 weeks of gestation.

Sampling. The umbilical cord was clamped and cut within the first 3 min after birth and prior to delivery of the placenta. Mixed arterial and venous cord blood was allowed to run freely, contamination with maternal blood being carefully avoided. The cord blood was stored at 4°C for no longer than 12 hours before serum was separated (2 000 r p m, 20 min) and the analyses begun.

METHODS

Total cholesterol (T-C) and triglyceride (TG) were determined as previously described by Andersen & Gry Nielsen (5). (VLDL+LDL)-cholesterol was measured in the following way: 200 µl of cord serum was mixed with 2.0 ml 0.05 M $CaCl_2$ and 40 µl 1% heparin (1 560 U/ml) at 20–30°C. After exactly 4 min the mixture was centrifuged for 10 min at 6000 r p m. The clear supernatant (HDL) was decanted, the tube left upside-down for 10

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AGA infants born between the ≥37th≤42th week of gestation

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Only newborns without signs of perinatal asphyxia (i e no meconium stained amniotic fluid fetal heart rate above 120/min during delivery no circumflexion of the umbilical cord and 1 min AS>7) were chosen to avoid cases with TNH Otherwise no selection of the newborns was made If the birthweight was above the 10th percentile value for gestation the infant was classified as AGA If the birth weight was below the 10th percentile value for gestation the infant was classified as SGA

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1 950	37	F	1.96	0.41	0.68	0.87	0.89
1 950	37	M	2.21	0.72	0.50	0.99	1.55
2 300	38	M	1.54	0.51	0.49	0.54	1.10
2 350	38	F	1.44	0.62	0.11	0.71	1.33
2 450	38	M	1.47	0.38	0.59	0.50	0.82
2 490	38	M	1.84	0.28	0.79	0.77	0.60
2 600	38	F	1.71	0.35	0.67	0.69	0.76
2 300	39	M	1.40	0.43	0.37	0.60	0.92
[illegible] 400	39	M	1.52	0.43	0.46	0.63	0.92
2 400	39	F	1.64	0.59	0.44	0.61	1.27
2 440	39	F	2.09	0.40	0.65	1.04	0.87
2 4[illegible]0	39	M	1.67	0.22	0.65	0.80	0.48
2 575	39	F	2.16	0.35	0.66	1.15	0.76
2 600	39	M	1.85	0.40	0.59	0.86	0.85
2 600	39	M	1.36	0.60	0.37	0.39	1.30
2 610	39	M	2.30	0.[illegible]8	0.76	1.26	0.61
2 650	39	M	1.[illegible]0	0.33	0.40	0.47	0.71
2 700	39	F	1.66	0.39	0.54	0.73	0.83
1 730	40	F	1.42	0.32	0.48	0.62	0.68
2 450	40	M	1.37	0.55	0.25	0.57	1.18
2 500	40	F	2.11	0.38	0.73	1.00	0.82
2 500	40	F	2.47	0.31	1.03	1.13	0.66
2 550	40	F	2.23	0.33	0.93	0.97	0.71
2 650	40	F	1.84	0.40	0.52	0.92	0.85
2 700	40	F	2.10	0.22	0.88	1.00	0.48
2 700	40	F	1.94	0.[illegible]0	0.57	1.17	0.44
2 750	40	M	1.91	0.30	0.65	0.96	0.65
2 750	40	M	1.60	0.45	0.59	0.56	0.96
2 800	40	F	2.22	0.66	0.81	0.75	1.42
[illegible] 800	40	M	1.60	0.62	0.25	0.73	1.34
2 800	40	F	2.20	0.40	0.79	1.01	0.85
2 750	41	F	2.16	0.76	0.67	0.73	1.63
2 600	42	M	1.86	0.37	0.46	1.03	0.80
2 700	42	F	2.20	0.31	0.75	1.14	0.67
10th percentile							
1 950	38		1.38	0.26	0.32	0.52	0.55
Median							
2 575	39		1.84	0.39	0.59	0.77	0.83
90th percentile							
2 770	40		2.22	0.64	0.84	1.14	1.37

Pregnant mothers entering the Rigshospital in Copenhagen in labor before the 37th week of pregnancy were given 12 mg of betamethasone i.m. immediately and this was repeated once 24 hours later. In addition 100 mg of Ritodrine in 1000 ml of 5.5% glucose was given i.v. over a 24 hour period and 100 mg of peroral phenobarbital divided into 3 doses was given daily until birth had taken place.

In the present study no matching was done between pairs of treated and untreated AGA infants <37 weeks of gestation.

Sampling. The umbilical cord was clamped and cut within the first 3 min after birth and prior to delivery of the placenta. Mixed arterial and venous cord blood was allowed to run freely, contamination with maternal blood being carefully avoided. The cord blood was stored at 4°C for no longer than 12 hours before serum was separated (2000 r.p.m., 20 min) and the analyses begun.

METHODS

Total cholesterol (T-C) and triglyceride (TG) were determined as previously described by Andersen & Gry Nielsen (5). (VLDL+LDL)-cholesterol was measured in the following way: 200 µl of cord serum was mixed with 2.0 ml 0.025 M $CaCl_2$ and 40 µl 1% heparin (1 560 U/ml) at 20–30°C. After exactly 4 min the mixture was centrifuged for 10 min at 6000 r.p.m. The clear supernatant (HDL) was decanted, the tube left upside-down for 10

Table 3 *Cord serum lipid and lipoprotein cholesterol concentrations in mmol/l in 16 purely premature newborns*

AGA infants born between the ≥33th<37th week of gestation not treated with betamethasone

Birth wt (g)	Gest age	Sex	T C	VLDL-C	LDL-C	HDL-C	TG
1 570	33	F	2.65	0.24	1.41	1.00	0.51
1 570	33	M	2.79	0.44	1.33	1.02	0.95
1 700	33	M	3.04	0.20	1.47	1.37	0.44
1 750	33	F	2.18	0.18	1.18	0.82	0.39
1 850	34	F	3.14	0.20	1.50	1.44	0.43
2 050	34	M	3.51	0.40	1.54	1.57	0.86
2 150	34	F	2.94	0.13	1.58	1.23	0.28
2 160	34	M	2.13	0.16	1.07	0.90	0.34
2 275	34	M	3.39	0.19	1.46	1.74	0.40
2 360	36	F	2.85	0.13	1.25	1.47	0.27
2 440	36	F	2.83	0.14	1.22	1.47	0.30
2 450	36	F	2.20	0.29	1.14	0.77	0.63
2 500	36	F	2.50	0.21	1.10	1.19	0.45
2 550	36	M	2.60	0.18	1.10	1.32	0.38
2 750	36	M	2.36	0.17	1.07	1.12	0.37
2 800	36	F	3.16	0.19	1.67	1.30	0.40
10th percentile							
1 570	33		2.16	0.13	1.07	0.80	0.28
Median							
2 217	34		2.81	0.19	1.29	1.26	0.40
90th percentile							
2 765	36		3.43	0.41	1.61	1.62	0.89

min and the precipitate (VLDL+LDL) redissolved in 200 μl 1.7% NaCl solution and the concentration of cholesterol determined LDL-cholesterol was calculated from the formula LDL-cholesterol (mmol/l)=(VLDL+LDL)-cholesterol (mmol/l) − TG (mmol/l)/2.15 HDL cholesterol was calculated from the formula HDL cholesterol (mmol/l)=total cholesterol (mmol/l) − (VLDL+LDL) cholesterol (mmol/l)

Statistics Percentile values were calculated to characterize the distribution of the different lipid and lipoprotein-cholesterol values For comparison of cord serum lipid and lipoprotein-cholesterol values among newborns with different gestational age the Mann Whitney test was used as described by Siegel (6)

RESULTS

In Tables 1–3 cord serum lipid and lipoprotein cholesterol values are given for AGA and SGA infants born after the 37th week of gestation and for AGA *infants born before the 37th week* of gestation

In Table 4 cord serum lipid and lipoprotein cholesterol values are given for AGA betamethasone treated infants born before the 37th week of gestation

In Table 5 a comparison is made between these groups and it is seen that the concentration of cord serum VLDL cholesterol and triglyceride is significantly higher in SGA infants compared with AGA infants born after the 37th week of gestation

A comparison between AGA infants born before and after the 37th week of gestation shows significantly higher values of total cholesterol LDL-cholesterol and HDL cholesterol in AGA *infants <37th week*

The betamethasone treated AGA infants <37 weeks are seen to have significantly higher total cholesterol and HDL cholesterol than AGA infants <37 weeks who had not been treated

DISCUSSION

The finding in the present study of significantly higher concentration of cord serum triglyceride in SGA infants compared with AGA infants born after the 37th week of gestation is

Table 4 *Cord serum lipid and lipoprotein cholesterol concentrations in mmol/l in 10 beta methasone treated purely premature newborns*

AGA infants born between the ≥33th<37th week of gestation

Birth wt (g)	Gest age	Sex	T-C	VLDL-C	LDL-C	HDL-C	TG
1 610	33	F	4 [illegible]1	0 14	2 23	1 84	0 30
1 830	33	F	4 40	0 14	1 94	2 3[illegible]	0 30
2 080	33	F	3 11	0 18	0 99	1 94	0 39
[illegible] 100	33	M	2 97	0 26	1 41	1.30	0.55
[illegible] 435	33	M	3 74	0 [illegible]2	0 99	2.53	0 48
1 970	34	M	4 10	0 13	1 9[illegible]	[illegible] 05	0.27
[illegible] 1 [illegible]0	34	M	3 74	0 15	1 2[illegible]	2 37	0 33
[illegible] 060	35	F	3 03	0 16	1.31	1.56	0 35
[illegible] 060	35	M	3 05	0 13	1 [illegible]7	1 65	0 28
[illegible] 110	35	M	3.86	0 14	1 49	[illegible] 23	0 31
10th percentile 1 63	33		2 98	0 13	0 99	1.3[illegible]	0 [illegible]7
Median [illegible] 0[illegible]0	33 5		3 74	0 14	1 36	1 99	0.3[illegible]
90th percentile [illegible] 403	35		4.38	0 [illegible]6	2 [illegible]0	[illegible] 51	0.54

in agreement with the findings of Tsang et al (3) Fosbrooke & Wharton (7) as well as Andersen & Friis Hansen (4)

The cause of the association between IUGR and elevated cord serum triglyceride remains speculative Christensen (8) found elevated cord serum FFA values in infants born after IUGR This might indicate an increased lipolysis in these fetuses and infants but as long as the minute volume of the placenta and the exchange of FFA across the placenta cannot be measured in humans the question remains unanswered although the first step to elucidating this important question has been taken by Hull (9) who demonstrated a rapid exchange of [1 ^{14}C]palmitate from the mother to the fetus In contrast to the present study Fosbrooke & Wharton (7) found significantly higher cord plasma triglyceride in full term than in preterm newborns

As for total cholesterol Rafstedt (10) has previously reported that cord plasma total cholesterol concentration was the same in infants with a birth weight below and above 2 500 g However he did not assess the gestational age of the infants and thus did not differentiate between AGA and SGA infants

Fosbrooke & Wharton (7) did not find any

Table 5 *Comparison of cord serum lipid and lipoprotein cholesterol concentrations in full term SGA full term AGA infants and preterm AGA infants not treated and treated with beta methasone*

	SGA≥37 weeks median values (mmol/l)		AGA≥37 weeks median values (mmol/l)		AGA<37 weeks median values (mmol/l)		Betamethasone treated AGA<37 weeks median values (mmol/l)
T-C	1 84	$p>0\ 05$	1 75	$p<0\ 001$	2 81	$p<0\ 01$	3 74
VLDL-C	0 39	$p<0\ 001$	0 [illegible]0	$p>0\ 05$	0 19	$p>0\ 05$	0 14
LDL-C	0 59	$p>0\ 05$	0.53	$p<0\ 001$	1 29	$p>0\ 05$	1 36
HDL-C	0 77	$p<0\ 01$	0 97	$p<0\ 01$	1 26	$p<0\ 001$	1 99
TG	0 83	$p<0\ 001$	0 4	$p>0\ 05$	0 40	$p>0\ 05$	0 3

Table 3 *Cord serum lipid and lipoprotein cholesterol concentrations in mmol/l in 16 purely premature newborns*

AGA infants born between the ≥33th<37th week of gestation not treated with betamethasone

Birth wt (g)	Gest age	Sex	TC	VLDL-C	LDL-C	HDL-C	TG
1 570	33	F	2.65	0.24	1.41	1.00	0.51
1 570	33	M	2.79	0.44	1.33	1.02	0.95
1 700	33	M	3.04	0.20	1.47	1.37	0.44
1 750	33	F	2.18	0.18	1.18	0.82	0.39
1 850	34	F	3.14	0.20	1.50	1.44	0.43
2 050	34	M	3.51	0.40	1.54	1.57	0.86
2 150	34	F	2.94	0.13	1.58	1.23	0.28
2 160	34	M	2.13	0.16	1.07	0.90	0.34
2 275	34	M	3.39	0.19	1.46	1.74	0.40
2 360	36	F	2.85	0.13	1.25	1.47	0.27
2 440	36	F	2.83	0.14	1.22	1.47	0.30
2 450	36	F	2.20	0.29	1.14	0.77	0.63
2 500	36	F	2.50	0.21	1.10	1.19	0.45
2 550	36	M	2.60	0.18	1.10	1.32	0.38
2 750	36	M	2.36	0.17	1.07	1.12	0.37
2 800	36	F	3.16	0.19	1.67	1.30	0.40
10th percentile							
1 570	33		2.16	0.13	1.07	0.80	0.28
Median							
2 217	34		2.81	0.19	1.29	1.26	0.40
90th percentile							
2 765	36		3.43	0.41	1.61	1.62	0.89

min and the precipitate (VLDL+LDL) redissolved in 200 µl 1.7% NaCl solution and the concentration of cholesterol determined. LDL cholesterol was calculated from the formula: LDL-cholesterol (mmol/l)=(VLDL+LDL)-cholesterol (mmol/l)−TG (mmol/l)/2.15. HDL cholesterol was calculated from the formula: HDL cholesterol (mmol/l)=total cholesterol (mmol/l)−(VLDL+LDL) cholesterol (mmol/l).

Statistics. Percentile values were calculated to characterize the distribution of the different lipid and lipoprotein-cholesterol values. For comparison of cord serum lipid and lipoprotein-cholesterol values among newborns with different gestational age, the Mann Whitney test was used as described by Siegel (6).

RESULTS

In Tables 1–3 cord serum lipid and lipoprotein cholesterol values are given for AGA and SGA infants born after the 37th week of gestation and for AGA infants born before the 37th week of gestation.

In Table 4 cord serum lipid and lipoprotein cholesterol values are given for AGA betamethasone treated infants born before the 37th week of gestation.

In Table 5 a comparison is made between these groups and it is seen that the concentration of cord serum VLDL cholesterol and triglyceride is significantly higher in SGA infants compared with AGA infants born after the 37th week of gestation.

A comparison between AGA infants born before and after the 37th week of gestation shows significantly higher values of total cholesterol, LDL cholesterol and HDL cholesterol in AGA infants <37th week.

The betamethasone treated AGA infants <37 weeks are seen to have significantly higher total cholesterol and HDL cholesterol than AGA infants <37 weeks who had not been treated.

DISCUSSION

The finding in the present study of significantly higher concentration of cord serum triglyceride in SGA infants compared with AGA infants born after the 37th week of gestation is

Table 4 *Cord serum lipid and lipoprotein cholesterol concentrations in mmol/l in 10 beta methasone treated purely premature newborns*

AGA infants born between the ⩾33th<37th week of gestation

Birth wt (g)	Gest age	Sex	T-C	VLDL-C	LDL-C	HDL-C	TG
1 610	33	F	4 ?1	0 14	? 23	1 84	0.30
1 830	33	F	4 40	0 14	1 94	? 32	0 30
2 080	33	F	3 11	0 18	0 99	1 94	0 39
? 100	33	M	2 97	0 _6	1 41	1 30	0.55
? 435	33	M	3 74	0 22	0 99	2 53	0 48
1 9?0	34	M	4 10	0 13	1 9?	2 05	0 ?7
? 170	34	M	3 74	0 15	1 2?	2 37	0 33
? 060	35	F	3 03	0 16	1.31	1.36	0 35
? 060	35	M	3 05	0 13	1 27	1 65	0 28
2 110	35	M	3 86	0 14	1 49	2 23	0.31
10th percentile 1 63	33		2 98	0 13	0 99	1.33	0 27
Median ? 070	33 5		3 74	0 14	1 36	1 99	0 32
90th percentile ? 403	35		4.38	0 ?6	2 ?0	2.51	0.54

in agreement with the findings of Tsang et al (3) Fosbrooke & Wharton (7) as well as Andersen & Friis Hansen (4)

The cause of the association between IUGR and elevated cord serum triglyceride remains speculative Christensen (8) found elevated cord serum FFA values in infants born after IUGR This might indicate an increased lipolysis in these fetuses and infants but as long as the minute volume of the placenta and the exchange of FFA across the placenta cannot be measured in humans the question remains unanswered although the first step to elucidating this important question has been taken by Hull (9) who demonstrated a rapid exchange of [1 ^{14}C]palmitate from the mother to the fetus In contrast to the present study Fosbrooke & Wharton (7) found significantly higher cord plasma triglyceride in full term than in preterm newborns

As for total cholesterol Rafstedt (10) has previously reported that cord plasma total cholesterol concentration was the same in infants with a birth weight below and above 2 500 g However he did not assess the gestational age of the infants and thus did not differentiate between AGA and SGA infants

Fosbrooke & Wharton (7) did not find any

Table 5 *Comparison of cord serum lipid and lipoprotein cholesterol concentrations in full term SGA full term AGA infants and preterm AGA infants not treated and treated with beta methasone*

	SGA⩾37 weeks median values (mmol/l)		AGA⩾37 weeks median values (mmol/l)		AGA<37 weeks median values (mmol/l)		Betamethasone treated AGA<37 weeks median values (mmol/l)
T-C	1 84	$p>0\ 05$	1 75	$p<0\ 001$	? 81	$p<0\ 01$	3 74
VLDL-C	0.39	$p<0\ 001$	0 ?0	$p>0\ 05$	0 19	$p>0\ 05$	0 14
LDL-C	0.59	$p>0\ 05$	0.53	$p<0\ 001$	1 29	$p>0\ 05$	1 36
HDL-C	0 77	$p<0\ 01$	0 9?	$p<0\ 01$	1 ?6	$p<0\ 001$	1 99
TG	0 83	$p<0\ 001$	0 4?	$p>0\ 05$	0 40	$p>0\ 05$	0 32

Table 3 *Cord serum lipid and lipoprotein cholesterol concentrations in mmol/l in 16 purely premature newborns*

AGA infants born between the ≥33th<37th week of gestation not treated with betamethasone

Birth wt (g)	Gest age	Sex	T-C	VLDL-C	LDL-C	HDL C	TG
1 570	33	F	2 65	0 24	1 41	1 00	0 51
1 570	33	M	2 79	0 44	1 33	1 02	0 95
1 700	33	M	3 04	0 20	1 47	1 37	0 44
1 750	33	F	2 18	0 18	1 18	0 82	0 39
1 850	34	F	3 14	0 20	1 50	1 44	0 43
2 050	34	M	3 51	0 40	1 54	1 57	0 86
2 150	34	F	2 94	0 13	1 58	1 23	0 78
2 160	34	M	2 13	0 16	1 07	0 90	0 34
2 275	34	M	3 39	0 19	1 46	1 74	0 40
2 360	36	F	2 85	0 13	1 25	1 47	0 27
2 440	36	F	2 83	0 14	1 22	1 47	0 30
2 450	36	F	2 20	0 29	1 14	0 77	0 63
2 500	36	F	2 50	0 21	1 10	1 19	0 45
2 550	36	M	2 60	0 18	1 10	1 32	0 38
2 750	36	M	2.36	0 17	1 07	1 12	0 37
2 800	36	F	3 16	0 19	1 67	1 30	0 40
10th percentile							
1 570	33		2 16	0 13	1 07	0 80	0 28
Median							
2 217	34		2 81	0 19	1 29	1 26	0 40
90th percentile							
2 765	36		3 43	0 41	1 61	1 62	0 89

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Statistics Percentile values were calculated to characterize the distribution of the different lipid and lipoprotein-cholesterol values For comparison of cord serum lipid and lipoprotein-cholesterol values among newborns with different gestational age the Mann Whitney test was used as described by Siegel (6)

RESULTS

In Tables 1–3 cord serum lipid and lipoprotein cholesterol values are given for AGA and SGA infants born after the 37th week of gestation and for AGA infants born before the 37th week of gestation

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Acta Pædiatr Scand 66 361–365 1977

GONADOTROPIN RELEASING HORMONE (LH RH) AND HUMAN CHORIONIC GONADOTROPIN IN THE TREATMENT OF TWO BOYS WITH HYPOGONADOTROPHIC HYPOGONADISM

S KRABBE and N E SKAKKEBÆK

From Childrens Hospital Fuglebakken University Department of Paediatrics and Laboratory of Reproductive Biology University Department of Obstetrics & Gynaecology Rigshospitalet Copenhagen Denmark

ABSTRACT Krabbe S and Skakkebæk N E (Childrens Hospital Fuglebakken University Department of Paediatrics and Laboratory of Reproductive Biology University Department of Obstetrics & Gynaecology Rigshospitalet Copenhagen Denmark) Gonadotropin releasing hormone (LH RH) and human chorionic gonadotropin in the treatment of two boys with hypogonadotrophic hypogonadism Acta Paediatr Scand 66 361 1977.—Two brothers 16 and 14 years of age with hypogonadotrophic hypogonadism and anosmia were treated with subcutaneous injections of 200 μg gonadotropin-releasing hormone at 8-hour intervals for 4 weeks Serum FSH increased to the range of normal adult men but serum LH and serum testosterone showed little change and no clinical signs of pubertal development occurred Thereafter the 2 patients were given HCG for 11 months and a combination of HCG and HMG for a further 3 months In response to this treatment the serum testosterone levels increased to the range of normal adult men and a marked development of the secondary sex characteristics was seen

KEY WORDS Male hypogonadotrophic hypogonadism gonadotropin releasing hormone human chorionic gonadotropin

Hypogonadotrophic hypogonadism in association with hyposmia or anosmia is known as Kallman s syndrome (11 17) The basic hormonal defect of this type of hypogonadism seems to be the lack of a normal production of the gonadotropin releasing hormone (1 7 11 13 19) Administration of this hormone would thus appear to be a rational therapy Some reports are available concerning a variable effect of prolonged LH RH administration (7 8 10 18 19) Mortimer et al (10) have concluded that continuous treatment with LH RH may induce puberty in prepubertal boys

We have compared LH RH treatment with a combination therapy of HCG (human chorionic gonadotropin) and HMG (human menopausal gonadotropin) in two brothers with hypogonadotrophic hypogonadism and anosmia

PATIENTS

Case I

A 16-year-old boy was referred to the clinic on a count of failure of pubertal development The testes were not present in the scrotum at birth and remained undescended A trial with HCG had been made for 6 weeks at the age of 6 years but the gonads did not descend Orchidopexy was then performed on the right and on the left side at the age of 9 and 10 years respectively

On admission the boy was found to be 171 cm in height but was thin and infantile with no signs of puberty The penis was small the right testis was located in the inguinal canal and the left testis in the scrotum and both measured 1 cm

Otoneurological examination revealed total anosmia Bone age was 13 years 3 months X ray of sella turcica was normal TSH plasma cortisol and the chromosome complement were normal Serum FSH was low and serum LH unmeasurable A test dose of 100 μg of LH RH given intravenously gave a pituitary response with a threefold increase in FSH and an increase in LH from undetectable to barely detectable levels as shown in Table I Biopsy from the scrotal testis showed no signs of activity of the Leydig-cells and spermatogenesis was not initiated although gonocytes were present

difference in cord plasma total cholesterol concentration in AGA infants above (mean 2 52 mmol/l) and in AGA infants below (mean 2 50 mmol/l) 37 weeks of gestation

In contrast Šabata (11) found significantly higher total cholesterol values in umbilical vein blood in prematures weighing 2000–2500 g (mean 4 07 mmol/l) compared to full term newborns (mean 2 10 mmol/l)

To our knowledge this study is the first presentation of lipoprotein cholesterol values in AGA infants born before the 37th week of gestation Assuming that circulating LDL cholesterol is a storage depot of liver cholesterol as suggested by Brown & Goldstein (12) the higher values of LDL cholesterol in these infants might indicate that the need for LDL cholesterol produced by the liver and transported to peripheral tissues for membrane and hormone synthesis may be higher between the 33th–37th week of gestation than at term

Whether the higher values of HDL cholesterol in cord serum of betamethasone treated infants does reflect an increased synthesis of phospholipids which are also transported in the HDL molecules remains to be answered

ACKNOWLEDGEMENTS

This study was supported by grants from the Danish Heart Foundation the Semper Nutrition Fund and the Dagmar Marshall Fund

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Submitted Sept 6 1976
Accepted Dec 7 1976

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Acta Pædiatr Scand 66 361–365 1977

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Table 1 *Initial effect of 100 µg LH RH on FSH and LH injected intravenously in two brothers with hypogonadism and anosmia*

FSH and LH are expressed as mIU 2nd IRP HMG/ml serum N D = not detectable

Minutes after LH RH injection	Case I		Case II	
	FSH	LH	FSH	LH
0	3 8	N D	3 6	N D
20	8 7	3 1	10 4	3 0
40	12 7	2 7	13 1	3 0
60	12 1	2 5	14 2	3 0

Case II

The 14 year-old brother of case 1 also referred to the clinic because of infantilism At the age of 8 years he was treated with HCG for cryptorchidism without success and orchidopexy on both sides was performed

On admission he was found to be 161 cm in height and had normal body proportions He was infantile with no signs of puberty Both testes measured 1 cm³ and were located in the scrotum

Otoneurological examination revealed total anosmia Bone age was 12 years and 6 months X ray of sella turcica was normal TSH plasma cortisol and the chromosome complement were normal The LH RH test showed a response similar to that of his brother (Table 1) Testicular biopsy showed no signs of activity of the Leydig cells and spermatogenesis was not initiated although gonocytes were present

METHODS

LH RH treatment

Both patients were given subcutaneous injections of 200 µg of LH RH at intervals of 8 hours for 4 weeks Blood specimens of peripheral venous blood were taken repeatedly as shown in Figs 1 and 2 All samples were analysed for FSH LH and testosterone In order to evaluate a possible long term effect the same analyses were carried out 10 weeks after the conclusion of treatment

HCG HMG treatment

Five months after withdrawal of LH RH therapy both boys were given a dose of 3 000 i u of HCG three times weekly administered as intramuscular injections Two months after this treatment was initiated both patients unexpectedly stopped taking the injections for 2 weeks because of fear of a too rapid pubertal development The regime was therefore continued with a HCG-dose of 3 000 i u twice weekly HCG was given alone for 11 months then HMG was added in a dose of 150 units three times weekly for 3 months and during that period the HCG dose was reduced to 1 500 i u three times weekly All treatment was then discontinued During the treatment regular clinical evaluations were performed and blood specimens for analysis of testosterone were taken once a month

Hormone assays

Serum FSH and LH were measured by specific radioimmuno assays (Statens Seruminstitut Copenhagen) The results are expressed in terms of the second international reference preparation of human menopausal gonadotropin (2nd IRP HMG per l serum) LER 907 (NIH) was used as standard preparation In these assays 1 mg LER 907 is equivalent to 200 i u 2nd IRP HMG of LH and 33 i u 2nd IRP HMG of FSH respectively (14)

Serum testosterone was measured by radioimmuno assay including thin layer chromatography (1?) (Medicinsk Laboratorium Copenhagen)

RESULTS

LH RH treatment

The variations in FSH LH and testosterone during treatment of both patients with LH RH are shown in Figs 1 2 and 3 Generally there was considerable fluctuation The levels of FSH showed a threefold increase during the first and the eighth day of therapy and in both patients all peak values were within the range of untreated normal adult men (14) The increment increased from the first to the eighth day after which no further increase was seen

The levels of LH were low during all 4 weeks In the first patient LH increased during the first day from undetectable to barely detectable levels thus implying a response

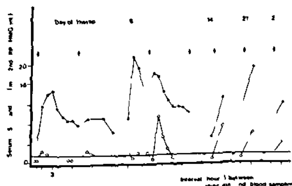


Fig 1 Serum FSH and serum LH values during daily treatment with LH RH given subcutaneously at 8 hour intervals for 28 days in a patient with hypogonadotrophic hypogonadism and anosmia (case I) *N D = not detectable*

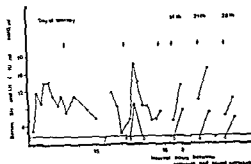


Fig 2 Serum FSH and serum LH values during daily treatment with LH RH given subcutaneously at 8 hour intervals for 28 days in a patient with hypogonadotrophic hypogonadism and anosmia (case II) N D =not detectable

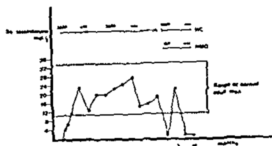


Fig 4 Serum testosterone values during combined HCG HMG treatment for 14 months in a patient with hypogonadotrophic hypogonadism and anosmia (case I)

within the prepubertal range (4 7) A further small increase was seen during the following days In the second patient LH remained undetectable during the first day but otherwise the responses were mostly similar to those of the first patient Both LH and FSH fell to very low levels shortly after the LH RH injections

Also the serum testosterone levels remained very low as shown in Fig 3 The serum testosterone value increased only slightly after injection of LH RH and in both cases the serum testosterone level was below the range found in early puberty in boys (2)

No change in the secondary sex characteristics was observed during the LH RH treatment

HCG HMG treatment

The serum testosterone concentrations during treatment with HCG and HMG are shown in Figs 4 and 5 Approximately 4 weeks after initiation of treatment the testosterone levels in both patients reached the lower limit of the range of normal adult men A marked drop in serum testosterone was found when treatment was withdrawn Normal adult levels of testosterone were obtained after resuming the regime although the dose of HCG given was smaller Four weeks after termination of treatment the serum testosterone values were similar to those found in normal prepubertal boys

Wide variations in serum testosterone were seen during the last 3 months of treatment We offer no explanation for this phenomenon but since the half lives of HCG and testosterone are short (6 17) even minor irregularities in

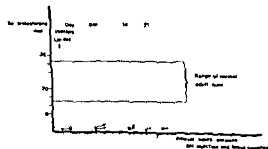


Fig 3 Serum testosterone values during daily treatment with LH RH given subcutaneously at 8 hour intervals for 28 days in two patients with hypogonadotrophic hypogonadism and anosmia O—O case I ●—● case II

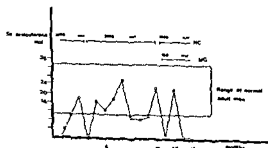


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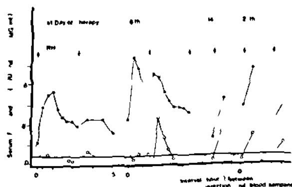


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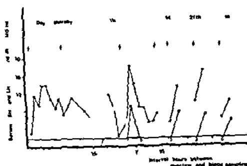


Fig 2 Serum FSH and serum LH values during daily treatment with LH RH given subcutaneously at 8 hour intervals for 28 days in a patient with hypogonadotrophic hypogonadism and anosmia (case II) N D = not detectable

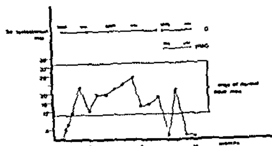


Fig 4 Serum testosterone values during combined HCG HMG treatment for 14 months in a patient with hypogonadotrophic hypogonadism and anosmia (case I)

within the prepubertal range (4 7) A further small increase was seen during the following days In the second patient LH remained undetectable during the first day but otherwise the responses were mostly similar to those of the first patient Both LH and FSH fell to very low levels shortly after the LH RH injections

Also the serum testosterone levels remained very low as shown in Fig 3 The serum testosterone value increased only slightly after injection of LH RH and in both cases the serum testosterone level was below the range found in early puberty in boys (2)

No change in the secondary sex characteristics was observed during the LH RH treatment

HCG HMG treatment

The serum testosterone concentrations during treatment with HCG and HMG are shown in Figs 4 and 5 Approximately 4 weeks after initiation of treatment the testosterone levels in both patients reached the lower limit of the range of normal adult men A marked drop in serum testosterone was found when treatment was withdrawn Normal adult levels of testosterone were obtained after resuming the regime although the dose of HCG given was smaller Four weeks after termination of treatment the serum testosterone values were similar to those found in normal prepubertal boys

Wide variations in serum testosterone were seen during the last 3 months of treatment We offer no explanation for this phenomenon but since the half lives of HCG and testosterone are short (6 17) even minor irregularities in

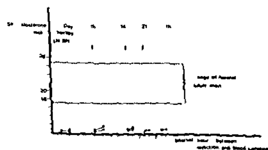


Fig 3 Serum testosterone values during daily treatment with LH RH given subcutaneously at 8 hour intervals for 28 days in two patients with hypogonadotrophic hypogonadism and anosmia O—O case I ●—● case II

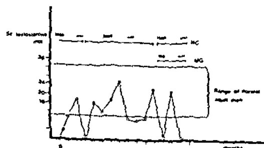


Fig 5 Serum testosterone values during combined HCG HMG treatment for 14 months in a patient with hypogonadotrophic hypogonadism and anosmia (case II)

the time of injection may cause considerable fluctuations in serum testosterone

Clinical marked development of the secondary sex characteristics was seen during the course of treatment Both boys reached a pubertal stage of grade IV (16) When the treatment was terminated in case I the right and left testis measured 6 and 3 ml, respectively, whereas the testicular volume increased to 3 ml on both sides in case II Both patients eventually experienced frequent erection and ejaculations, especially during the last 3 months Six weeks after the end of treatment it was not possible for the first patient to have ejaculation and the other had a small ejaculate

DISCUSSION

The findings in the present study support the theory that hypogonadotrophic hypogonadism may be caused by a primary hypothalamic defect involving a defective release or impaired synthesis of the gonadotropin releasing hormone These results are at variance with those of Mortimer et al (10) who concluded that long term treatment with LH RH may constitute an effective means of treating patients with hypogonadotrophic hypogonadism However the dose of LH RH and the period of treatment are not comparable in the two studies, which may account for the difference in the results Mortimer and co workers used a dose of 500 μg LH RH given as subcutanous injections at 8 hour intervals for 4 to 20 weeks in the prepubertal patients The administration of LH RH in our patients resulted in a release of gonatropins from the pituitary but this treatment did not induce serum levels of FSH and LH sufficient to stimulate the testes to produce adequate amounts of testosterone It is possible that the administration of LH RH over a longer period of time and/or in larger doses would have initiated puberty in our patients However the necessity of frequent injections made it impossible to continue administration of the hormone A recent report of a LH RH analogue with effect after oral administration is of great interest (5)

Patients with hypogonadotrophic hypogonadism usually show good testicular response to exogenous gonadotropins, though Bardin et al (3) reported that the response of some patients to HCG was poor Our patients showed testicular response with serum testosterone levels in the normal adult range and a marked development of secondary sex characteristics during the HCG HMG treatment

To conclude we failed to induce a significant increase in serum testosterone levels by 8 hourly subcutaneous injections of 200 μg LH RH for 4 weeks in two boys with hypogonadotrophic hypogonadism although both patients showed some response to LH RH by an increase in serum FSH and serum LH Further development of orally active or long acting injectable LH RH would seem to be essential if this hormone is to be used in the treatment of male hypogonadotrophic hypogonadism

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Acta Pædiatr Scand 66 367–372 1977

MEASLES AS A CAUSE OF FETAL DEFECTS

A Retrospective Study of Ten Measles Epidemics in Greenland

CARSTEN SAND JESPERSEN JØRGEN LITTAUER and UFFE SAGILD

From Dronning Ingrids Hospital Medical Department Godthaab Greenland

ABSTRACT Sand Jespersen C Littauer J and Sagild U (Dronning Ingrids Hospital Godthaab Greenland) Measles as a cause of fetal defects A retrospective study of ten measles epidemics in Greenland Acta Paediatr Scand 66 367 1977 —In a retrospective study of ten epidemics of measles in virgin-soil populations in Greenland 368 women were found to be pregnant at the time of their infection with measles Information on the course of the pregnancies was obtained in 327 of these women and a clinical examination was made of 252 of their children The risk of fetal death among women infected in the first trimester was found to be high About half of 20 women infected during their first two months of pregnancy and a fifth of 31 women infected in the third month had abortions 9% of 64 women infected in the first trimester and going to term had stillbirths 28 women infected in the first two months of pregnancy had live children but four of these had congenital malformations three of extreme rarity and severity leading to death The rate of perinatal mortality and prematurity was equal among infants exposed to measles in the first second and third trimester of fetal life

KEY WORDS Measles fetal defects fetal death congenital malformations perinatal mortality prematurity Greenland

Since Gregg s epoch making report in 1941 (5) on the association between maternal rubella and fetal defects several reports on the possible effects of viral infections on fetal development have appeared The effect of measles on fetuses during early pregnancy has also been studied but the number of cases was too small to allow definite conclusions to be drawn (9 10) As the disease is very rare in the child bearing population of urban communities so called virgin soil populations have been the only source from which an adequate number of infected pregnant women could be obtained An early report on an epidemic of measles in South Greenland (3) suggested a significantly increased abortion rate in women infected early in pregnancy Later in a study of 155 pregnant women infected with measles during different epidemics in Greenland we suggested that measles in the first trimester of pregnancy involves a risk for the fetus probably comparable to that previously observed for rubella (7)

The present report concerns a retrospective study of the outcome of 327 women infected with measles The cases are collected from all registered epidemics of measles in virgin soil populations in Greenland—all occurring in the period 1951–1962 The 155 pregnancies reported previously are included

Four aspects of measles in pregnancy have been studied 1) Rate of fetal death 2) incidence of congenital defects in live born infants 3) infant mortality and 4) rate of prematurity

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Geographical localization	Year	Infected population	Infected pregnant women	No of pregnancies in this study
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Julianehaab	1951	893	36	29
Egedesminde	1954	1 054	37	29
Godthaab	1954	1 422	45	41
Holsteinsborg	1955	1 022	44[a]	39[b]
Sukkertoppen	1955	1 037	40	34[c]
Jacobshavn	1959	1 178	33	31
Umanak	1962	1 824	36	36
Egedesminde district	1962	1 806	37	28
Angmagssalik	1962	1 800	49	49
Total		12 473	368	327

Hospital records of abortions in these areas during the relevant period have not been found and may have been discarded
[b] One pair of twins
[c] Two pairs of twins

each epidemic and its effects on individual communities were submitted by local medical personnel to the central health authorities in Greenland (1 2 4)

In the majority of cases diagnoses were made by local physicians although in some areas specially trained nurses constituted the only medical authority available The diagnosis of measles was never in doubt in the reported group of patients

In Greenland all deliveries are registered in the local hospitals In addition it is customary to admit all cases with a history of suspected or verified abortion in the second month of gestation or later By studying this material we were able to indentify all women having an abortion or a delivery during the period from the beginning of each of the epidemics until 40 weeks after their termination

The date of conception calculated from the recorded date of expected delivery or the last menstrual period was compared with the date of appearance of the measles rash in each case and a number of women presumably infected before conception were indentified and excluded from the material A number of women who were infected after delivery were similarly excluded In this way we obtained a group of 368 women infected with measles during pregnancy who with the addition of a presumably small group on non registered cases of abortion (to be discussed later) would constitute all pregnant women infected during the epidemics mentioned (Table 1)

An attempt was made to localize and to contact all these women but in 41 cases we were unsuccessful because of deficient registers Thus the outcome of these pregnancies is unknown

Of the remaining 327 women 61 were not contacted by us because in addition to their living in geographical areas difficult to approach evidence was available to indicate that these women had either had an abortion or the exposed child had died at birth or later In the latter cases the causes of death were ascertained as far as possible through death certificates or in some cases through hospital records

All the remaining 266 women were contacted by one of us at which time the women s recollections regarding their measles infections and the course of their pregnancies were checked against previously obtained information

At the same time all surviving children exposed to measles during their intrauterine life 252 in all were examined

These children whose age at the time of the study varied between one and sixteen years underwent a general clinical examination including measurements of weight and height and in 150 cases standard electrocardiographic tracings were obtained Included in this group are all cases with cardiac murmurs or other signs of congenital heart disease In the remaining 10[?] cases with no suspicion of heart disease mainly living in remote areas with no access to electrocardiographic equipment it was considered that practical difficulties prohibited the taking of tracings

X ray studies of the chest were obtained in all cases of suspected cardiac disease In addition all X ray records obtained during previous annual check ups for tuberculosis (attended by practically all of the population were screened for evidence of cardiovascular disease

Finally to check the possible presence of congenital hearing defects all children aged twelve years or more at the time of the study (104 in all) were subjected to audiometric tests Circumstances did not permit the use of sound insulated rooms A hearing loss was considered to be present if the audiogram showed a loss of more than 2[?] db at one or more frequencies in one or both ears

RESULTS

1 *Rate of fetal death*

The rate of abortion by week of gestation at the time of the measles infection is shown in Table 2 The table includes only 214 pregnancies because hospital records of abortions during the relevant period in four epidemic areas could not be located and may have been discarded (cf note to Table 1) The table includes 51 cases of women at risk of abortion infected in the first trimester and 76 in the second It appears that about 30% infected in the first trimester had abortions while among those infected in the second trimester only 4% of the cases had abortions

Despite the uncertainty in relating the date of infection to the date of conception very early in pregnancy we believe that the data

Table 2 *Rate of abortion by week of gestation at time of infect on*

Fetal age at time of infection (weeks)	No of infected mothers	Abortions	
		No	%
1–4	6	4	67
5–8	14	6	43
9–13	31	6	19
1st trimester total	51	16	32
14–26	76	3	4
27–40	87		
Total	214	19	9

In tables 2 3 4 5 and 6 fetal age is derived from the calculated date of conception

Table 3 *Rate of stillbirth in relation to fetal age at time of measles infection*

Fetal age at time of infection (weeks)	No of children from non aborting infected mothers	Stillbirths	
		No	%
1–4	6	0	0
5–8	17	0	0
9–13	41	6	15
1st trimester total	64	6	9
14–26	114	3	3
27–40	133	2	2
Total	311	11	3

Including two pairs of twins

suggest the risk of abortion is greatest when the mother is infected in the first part of the first trimester and is probably about 50%

Table 3 shows the rate of stillbirth in relation to the fetal age at the time of infection With increasing fetal age at the time of infection the risk of stillbirth decreases but the difference between groups is not great It is noteworthy however that the women infected in the first two months despite high abortion risks did not have stillbirths

2 *Congenital malformations*

Table 4 lists eight cases of gross congenital malformations previously diagnosed among 300 liveborn infants

Among 58 infants with mothers infected in the first trimester five cases were found (9%) Three of these five died before the age of ten months by causes directly attributable to their defects These three infants were afflicted with malformations of extreme rarity One had an atypical cerebral leucodystrophy with dysplasia particularly in the right hemisphere the second was a cyclop with *microcephaly* and polydactyli and the third infant had *multiple* cardiac anomalies atrial septal defect atrophic right ventricel communicating through a ventricular septal defect with a hypertrophic left ventricle and a hypertrophic aorta total atresia of the tricuspid valve and *stenosis of* the pulmonary valve The other two children survived with defects of less rarity and severity (anal atresia and harelip)

Among 111 children of mothers infected in their second trimester of pregnancy there were two cases of known or presumed congenital malformations (spastic tetraplegia stenosis of pulmonary artery) Both children are alive

Finally among 131 children whose mothers

Table 4 *Congenital malformations among 300 liveborn infants*[a]

Case no	Sex	Fetal age at time of infection (month)	Diagnosis	Course
1	f	First	Atypical leucodystrophy of brain	Dead 10 weeks
2	f	Second	Cyclopia	Dead ? hours
3	m	Second	Multiple cardiac anomalies	Dead 10 months
4	m	Second	Anal atresia	Alive
5	f	Third	Cheilo-gnato palato-schisis	Alive
6	m	Fourth	Spastic tetraplegia	Alive
7	m	Fifth	Stenosis of pulm artery	Alive
8	f	Eight	Patent duct arteriosus	Alive

Complete case histories may be obtained on request

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The rate of abortion by week of gestation at the time of the measles infection is shown in Table 2 The table includes only 214 pregnancies because hospital records of abortions during the relevant period in four epidemic areas could not be located and may have been discarded (cf note to Table 1) The table includes 51 cases of women at risk of abortion infected in the first trimester and 76 in the second It appears that about 30% infected in the first trimester had abortions while among those infected in the second trimester only 4% of the cases had abortions

Despite the uncertainty in relating the date of infection to the date of conception very early in pregnancy we believe that the data

Table 2 *Rate of abortion by week of gestation at time of infection*

Fetal age at time of infection (weeks)	No of infected mothers	Abortions	
		No	%
1– 4	6	4	67
5– 8	14	6	43
9–13	31	6	19
1st trimester total	51	16	32
14–26	76	3	4
27–40	87		
Total	214	19	9

In tables 2 3 4 5 and 6 fetal age is derived from the calculated date of conception

Table 3 *Rate of stillbirth in relation to fetal age at time of measles infection*

Fetal age at time of infection (weeks)	No of children from non aborting infected mothers	Stillbirths	
		No	%
1– 4	6	0	0
5– 8	17	0	0
9–13	41	6	15
1st trimester total	64	6	9
14–26	114	3	3
27–40	133	2	2
Total	311	11	3

Including two pairs of twins

suggest the risk of abortion is greatest when the mother is infected in the first part of the first trimester and is probably about 50%

Table 3 shows the rate of stillbirth in relation to the fetal age at the time of infection With increasing fetal age at the time of infection the risk of stillbirth decreases but the difference between groups is not great It is noteworthy however that the women infected in the first two months despite high abortion risks did not have stillbirths

2 *Congenital malformations*

Table 4 lists eight cases of gross congenital malformations previously diagnosed among 300 liveborn infants

Among 58 infants with mothers infected in the first trimester five cases were found (9%) Three of these five died before the age of ten months by causes directly attributable to their defects These three infants were afflicted with malformations of extreme rarity One had an atypical cerebral leucodystrophy with dysplasia particularly in the right hemisphere the second was a cyclop with microcephaly and polydactyli and the third infant had multiple cardiac anomalies atrial septal defect atrophic right ventricel communicating through a ventricular septal defect with a hypertrophic left ventricle and a hypertrophic aorta total atresia of the tricuspid valve and stenosis of the pulmonary valve The other two children survived with defects of less rarity and severity (anal atresia and harelip)

Among 111 children of mothers infected in their second trimester of pregnancy there were two cases of known or presumed congenital malformations (spastic tetraplegia stenosis of pulmonary artery) Both children are alive

Finally among 131 children whose mothers

Table 4 *Congenital malformations among 300 liveborn infants*

Case no	Sex	Fetal age at time of infection (month)	Diagnosis	Course
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2	f	Second	Cyclopia	Dead 2 hours
3	m	Second	Multiple cardiac anomalies	Dead 10 months
4	m	Second	Anal atresia	Alive
5	f	Third	Cheilo-gnato palato-schisis	Alive
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Complete case histories may be obtained on request

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Godthaab	1954	1 422	45	41
Holsteinsborg	1955	1 022	44	39[b]
Sukkertoppen	1955	1 037	40	34
Jacobshavn	1959	1 178	33	31
Umanak	1962	1 824	36	36
Egedesminde district	1962	1 806	37	28
Angmagssalik	1962	1 800	49	49
Total		*12 473*	*368*	*327*

[a] Hospital records of abortions in these areas during the relevant period have not been found and may have been discarded
[b] One pair of twins
[c] Two pairs of twins

each epidemic and its effects on individual communities were submitted by local medical personnel to the central health authorities in Greenland (1 2 4)

In the majority of cases diagnoses were made by local physicians although in some areas specially trained nurses constituted the only medical authority available The diagnosis of measles was never in doubt in the reported group of patients

In Greenland all deliveries are registered in the local hospitals In addition it is customary to admit all cases with a history of suspected or verified abortion in the second month of gestation or later By studying this material we were able to indentify all women having an abortion or a delivery during the period from the beginning of each of the epidemics until 40 weeks after their termination

The date of conception calculated from the recorded date of expected delivery or the last menstrual period was compared with the date of appearance of the measles rash in each case and a number of women presumably infected before conception were indentified and excluded from the material A number of women who were infected after delivery were similarly excluded In this way we obtained a group of 368 women infected with measles during pregnancy who with the addition of a presumably small group on non registered cases of abortion (to be discussed later) would constitute all pregnant women infected during the epidemics mentioned (Table 1)

An attempt was made to localize and to contact all these women but in 41 cases we were unsuccessful because of deficient registers Thus the outcome of these pregnancies is unknown

Of the remaining 327 women 61 were not contacted by us because in addition to their living in geographical areas difficult to approach evidence was available to indicate that these women had either had an abortion or the exposed child had died at birth or later In the latter cases the causes of death were ascertained as far as possible through death certificates or in some cases through hospital records

All the remaining 266 women were contacted by one of us at which time the women s recollections regarding their measles infections and the course of their pregnancies were checked against previously obtained information

At the same time all surviving children exposed to measles during their intrauterine life 252 in all were examined

These children whose age at the time of the study varied between one and sixteen years underwent a general clinical examination including measurements of weight and height and in 150 cases standard electrocardiographic tracings were obtained Included in this group are all cases with cardiac murmurs or other signs of congenital heart disease In the remaining 10? cases with no suspicion of heart disease mainly living in remote areas with no access to electrocardiographic equipment it was considered that practical difficulties prohibited the taking of tracings

X ray studies of the chest were obtained in all cases of suspected cardiac disease In addition all X ray records obtained during previous annual check ups for tuberculosis (attended by practically all of the population) were screened for evidence of cardiovascular disease

Finally to check the possible presence of congenital hearing defects all children aged twelve years or more at the time of the study (104 in all) were subjected to audiometric tests Circumstances did not permit the use of sound insulated rooms A hearing loss was considered to be present if the audiogram showed a loss of more than 20 db at one or more frequencies in one or both ears

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the rapidly increasing development of com munications with previously virgin soil pop ulations it is not likely that opportunities for prospective studies of groups of similar size will arise

The difficulties inherent in the acquisition of valid data on fetal death early in pregnancy are obvious (10) Women are usually not seen for prenatal care until late in the first trimester and some cases of abortions may pass unrec ognized or remain unreported Thus exact figures demonstrating the increased risk of abortion following infection with measles in early pregnancy are difficult to obtain

In the present paper there is a considerable difference between the numbers of women pregnant in the first second and third trimes ter at the time of infection and in the first trimester between the numbers of those in fected in the first second and third month of gestation (Tables 2 3 5 6) Possibly the pres ence of an epidemic in a community may reduce the number of conceptions during this period Furthermore the method used in this paper to calculate the dates of conception and infection may have resulted in the exclusion of some women because this calculation errone ously indicated the date of infection before the date of conception We thus believe that the data for the rates of abortion shown in Table 2 represent minimum figures

The risk of fetal death among women in fected in the first trimester is high Half of the women infected in the first two months of ges tation and one out of five of those infected in the third month had abortions Among the re maining women going to term one in seven had a dead child

23 women infected with measles in the first two months of pregnancy gave birth to live children but four of these had congenital malformations Three of the congenital defects were of extreme rarity and severity leading directly to death It is hardly conceivable that the measles infection as such was not a domi nant etiological factor in these cases of con genital malformations

The remaining four of the eight cases of congenital malformations found in the total group of 300 children were of much more common types and the children were born by mothers infected later in pregnancy As the incidence of congenital malformations in Greenland is known to be high (6) the measles infection may not have been an etiological factor in these cases The audiometric tests revealed abnormal hearing in about 25% of the children examined In most of these cases this could be attributed to previous middle ear infection which is known to be a common cause of hearing defects in Greenland (8) We believe that our data on the remaining children are too sparse to allow conclusions regarding the etiology of the hearing loss

The perinatal mortality figures are more dif ficult to interpret We did not as expected find any significantly higher perinatal mortal ity among children exposed to measles early in fetal life but the mortality among the liveborn infants as a whole irrespective of the time of exposure to measles was higher than normal in Greenland (about 10% versus approx 7%) (11) Information on the causes of death of the 32 children dying in the first year of life is fragmentary Possibly the high mortality among those infected early in fetal life may partly be due to a decreased viability caused by the maternal infection while the high mor tality among those exposed towards the end of gestation may be due to external factors in the environment into which they were born We know of a few cases of early death attributed to the mother being sick with measles or com plications thereof at the time of delivery

Regarding prematurity the findings were similar There was no significant difference in the rate of prematurity between the groups of children exposed in the first second or third trimester but the rate of prematurity as a whole was somewhat higher than is usual in Greenland (4) Again it is conceivable that some children exposed to measles in the first part of their intrauterine life were small at birth because of some inherent damage caused by

Table 5 *Infant mortality in relation to fetal age at time of maternal measles infection*

Fetal age at time of infection (weeks)	No of liveborn infants	Infant mortality during first year of life	
		No	%
1–4	5	2	40
5–8	14	2	14
9–13	34	2	5
1st trimester total	53	6	11
14–26	107	10	9
27–40	126	16	13
Total	286	32	12

were infected in their third trimester of pregnancy, only one child with a congenital malformation a case of patient ductus arteriosus was diagnosed The diagnosis was verified in a successfully performed operation

Among 252 children exposed to measles during their intrauterine life no further cases with definite evidence of congenital malformations were found 27 children were found to have cardiac murmurs which were however soft and systolic in character all had normal electrocardiograms and chest X rays and none had symptoms suggesting cardiac disease Electrocardiographic abnormalities were found in five children without any associated signs or symptoms of cardiac disease One child had a complete right bundle branch block (RBB) two had incomplete RBB and one had a grade one a v block

Audiometric tests revealed abnormal hearing in 27 cases of 104 studied In 17 instances the hearing loss could be attributed to previous middle ear infection Among the remaining ten seven had significant hearing loss only at high frequencies (8 000 Hz or 4 000+8 000 Hz) Finally three children whose mothers were infected by measles during their 4th 5th and 9th month respectively had bilateral diffuse hearing loss of about 40–50 db

3 *Infant mortality*

Table 5 relates the infant mortality to the stage of gestation at the time of the maternal infection From the total group of 300 liveborn infants we have excluded the previously mentioned 8 cases of established congenital malformations and three pairs of twins In fact, two of the eight children with congenital malformations and two pairs of the twins died during their first year of life

Although the data might suggest a decreased viability among those exposed to measles early during their intrauterine life the figures are too small to allow any definite conclusions

4 *Rate of prematurity*

Information on the birth weights of 231 infants exposed to maternal measles was available Two pairs of twins have been excluded from this group The birth weight in relation to the stage of gestation at the time of the maternal infection is shown in Table 6 26 children (11%) had birth weights below 2 500 grams The birth weight appears to be uninfluenced by the stage of gestation at the time of infection

DISCUSSION

The present study was conducted retrospectively which imposes obvious drawbacks compared to the prospective method It is however by far the largest group of measles infected pregnant women reported and with

Table 6 *Low birth weight of liveborn infants in relation to fetal age at time of maternal measles infection*[a]

Fetal age at time of infection (weeks)	No of liveborn infants	Low birth weight of infants	
		No	%
1–4	9	2	22
5–8	14	2	14
9–13	32	2	6
1st trimester total	55	6	11
14–26	80	11	14
27–40	92	9	10
Total	227	26	11

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the rapidly increasing development of communications with previously virgin soil populations it is not likely that opportunities for prospective studies of groups of similar size will arise

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Regarding prematurity the findings were similar There was no significant difference in the rate of prematurity between the groups of children exposed in the first second or third trimester but the rate of prematurity as a whole was somewhat higher than is usual in Greenland (4) Again it is conceivable that some children exposed to measles in the first part of their intrauterine life were small at birth because of some inherent damage caused by

the maternal infection while the children ex posed later in gestation were small because their mothers gave birth prematurely as a re sult of measles infection However we have no data to substantiate this point

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Submitted Sept 14 1976
Accepted Oct 30 1976

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Acta Pædiatr Scand 66 373–379 1977

SEVERE MENTAL RETARDATION IN A SWEDISH COUNTY

I *Epidemiology Gestational Age Birth Weight and Associated CNS Handicaps in Children Born 1959–70*

K H GUSTAVSON B HAGBERG G HAGBERG and K SARS

From the Departments of Paediatrics Universities of Gothenburg Umeå and Uppsala Sweden

ABSTRACT Gustavson K H Hagberg B Hagberg G and Sars K (Departments of Paediatrics Universities of Gothenburg Umeå and Uppsala) Severe mental retardation in a Swedish county I Epidemiology gestational age birth weight and associated CNS handicaps in children born 1959–70 Acta Paediatr Scand 66 373 1977.—In an unselected series of children born in 1959–70 with severe mental retardation (MR)—defined as IQ<50—in a Swedish county the incidence prevalence gestational length birth weight and associated CNS handicaps were analysed The cumulative incidence at 1–16 years of age was calculated at 3.3% and the prevalence at 11–16 years at 2 8‰ These figures were lower than in most other previous studies In the great majority of cases the pathogenesis was of prenatal origin The mean gestational lengths and birth weights were decreased compared with those of an average Swedish population Severe MR affected large and small for date babies more often than could be expected On the other hand babies with a low birth weight appropriate for gestational age and with an uncomplicated history were found not to run a special risk of severe MR Among the 122 children 42% had one or more associated CNS handicaps—epilepsy (30%) and cerebral palsy (18%) being the most common

KEY WORDS Severe mental retardation incidence prevalence birth weight associated CNS handicaps

In recent years doors have been opened for partial prevention of the birth of severely mentally retarded children Organized genetic counselling prenatal diagnosis of chromosomal aberrations hereditary biochemical disorders and neural tube defects vaccination of school girls against rubella and careful obstetric supervision of pregnancies at risk all together today ought to decrease the incidence of severe mental retardation (MR) (9) For comparative purposes in future epidemiological research it was thought to be of interest to sum up available data from severely mentally retarded children born in the period 1959–70 as this period may be considered to be the last one before the gradual introduction of prenatal prevention in Sweden and at the same time the period during which important peri and neonatal advances were documented (10)

This first part of the study deals with the incidence and prevalence of severe MR and the distribution according to sex gestational age birth weight and associated CNS handicaps

A second part will deal with the etiologic and pathogenetic aspects

MATERIALS AND METHODS

Definition

In this series severe mental retardation corresponds to an IQ<50 and present before the age of 18 years Apart from the most profoundly retarded children where the severity of their mental handicap was without doubt the children

the maternal infection while the children exposed later in gestation were small because their mothers gave birth prematurely as a result of measles infection However we have no data to substantiate this point

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ABSTRACT Gustavson K H Hagberg B Hagberg G and Sars K (Departments of Paediatrics Universities of Gothenburg Umeå and Uppsala) Severe mental retardation in a Swedish county I Epidemiology gestational age birth weight and associated CNS handicaps in children born 1959–70 Acta Paediatr Scand 66 373 1977.—In an unselected series of children born in 1959–70 with severe mental retardation (MR)—defined as IQ<50—in a Swedish county the incidence prevalence gestational length birth weight and associated CNS handicaps were analysed The cumulative incidence at 1–16 years of age was calculated at 3.3‰ and the prevalence at 11–16 years at 2.8‰ These figures were lower than in most other previous studies In the great majority of cases the pathogenesis was of prenatal origin The mean gestational lengths and birth weights were decreased compared with those of an average Swedish population Severe MR affected large and small for date babies more often than could be expected On the other hand babies with a low birth weight appropriate for gestational age and with an uncomplicated history were found not to run a special risk of severe MR Among the 122 children 42% had one or more associated CNS handicaps—epilepsy (30%) and cerebral palsy (18%) being the most common

KEY WORDS Severe mental retardation incidence prevalence birth weight associated CNS handicaps

In recent years doors have been opened for partial prevention of the birth of severely mentally retarded children Organized genetic counselling prenatal diagnosis of chromosomal aberrations hereditary biochemical disorders and neural tube defects vaccination of school girls against rubella and careful obstetric supervision of pregnancies at risk all together today ought to decrease the incidence of severe mental retardation (MR) (9) For comparative purposes in future epidemiological research it was thought to be of interest to sum up available data from severely mentally retarded children born in the period 1959–70 as this period may be considered to be the last one before the gradual introduction of prenatal prevention in Sweden and at the same time the period during which important peri and neonatal advances were documented (10)

This first part of the study deals with the incidence and prevalence of severe MR and the distribution according to sex gestational age birth weight and associated CNS handicaps

A second part will deal with the etiologic and pathogenetic aspects

MATERIALS AND METHODS

Definition

In this series severe mental retardation corresponds to an IQ<50 and present before the age of 18 years Apart from the most profoundly retarded children where the severity of their mental handicap was without doubt the children

Table 1 *Incidence of severe mental retardation in children aged 5–16 years born in the county of Uppsala Sweden in 1959–70 and alive at one year of age*

Birth year	Age	Number alive at one year of age	Severe mental retardation	
			n	‰
1959–64	11–16	16 779	55	3 28
1965–70	5–10	20 431	52	2 54
Total	5–16	37 210	107	2 88

had been given intelligence tests to determine suitability for education The tests used in most cases were the Terman–Merrill WISC and Merril-Palmer tests

Area investigated

The study covers a Swedish county the county of Uppsala with a population of slightly more than 200 000 inhabitants (1970) and of an average demographic structure There is one main central city Uppsala with a large teaching hospital and exceptionally good facilities for integrated neuropaediatric supervision and follow up of mentally retarded children

Search procedure

The object of the investigation was to trace every case of severe MR in children born 1959–70 in the county of Uppsala and alive at the age of one year This age restriction was made for two main reasons Firstly we considered it doubtful to retrospectively diagnose severe MR in a child who had died during its first year of life secondly the official statistics of Sweden give yearly the figures of live birth rates as well as the mortality during the first year of life

In each county of Sweden mentally retarded children are registered by a Board for Provisions and Services to the Mentally Retarded (BPSMR) (Omsorgsstyrelsen) with established laws and providing all sorts of support for daily life education training and habilitation Every child is dependent on being registered under this authority to get their full governmental and municipal support

As the present study was performed in 1975 the youngest children were at least 5 years old and it was highly probable that they all would be known by the above authority An examination was made of the register held by this authority for children born in 1959–70 in the county of Uppsala Children removed from the register because of death or migration were also noted Using the official ten digit registration number system alloted to everybody check ups were made in the registers of the same authorities in all other Swedish counties in order to trace those migrated before BPSMR registration but born in the Uppsala county The case records for MR at the departments of paediatrics and child psychiatry in Uppsala University Hospital for the years concerned were examined The death register at the departments of paediatrics the period 1960–75 was also scrutinized and those with established severe MR as well as with diagnoses known always to be connected with severe MR were included

The present series was considered complete due to the special medical insurance system in Sweden as well as the high degree of sociomedical organization and the well developed child health care system of this geographical area In addition since 1959 the medical service system for mentally retarded children has been centralized to Uppsala and intimately integrated with the neuropaediatric section of the department of paediatrics of the University Hospital

RESULTS AND CONCLUSIONS

A total of 122 children who were born in the county of Uppsala and satisfied the criteria of severe MR were traced The official registration of live births in Sweden is based on the mother s parish registration at the time of delivery Of the mothers 107 were registered in the county of Uppsala at the time of delivery and the number of children included in the incidence and prevalence calculations were therefore limited to 107 For the rest of the study all the 122 children were included

Incidence and prevalence

The investigation period 1959–70 was divided into the two periods 1959–64 and 1965–70 in order to study any changes in incidence The incidence of severely mentally retarded per 1 000 children alive at one year of age during the two periods is shown in Table 1 No statistically significant difference between the two periods was revealed The lower figure for 1965–70 may be accidental but it is more

Table 2 *Incidence of Down s syndrome among children live born in the county of Uppsala Sweden in 1959–70*

One child with IQ>50 included

Birth year	Number of live births	Down s syndrome	
		n	
1959–64	17 043	14	0 82
1965–70	20 689	19	0 92
Total	37 732	33	0 88
Including 40% dead during first year of life		55	1 46

Table 3 *Mean birth weight in different pathogenetic groups in a series of 122 children with severe MR born in 1959–70 FDS = Foetal deprivation of supply (7 11)*

Pathogenetic group	*n*	Mean birth weight (g)
Down s syndrome	39	3 106
Other genetic disorders	14	3 473
Other prenatal stigmata	24	3 795
Remaining patients with certain factors		
FDS	13	2 671
Asphyxia/cerebral haemorrhage	12	2 897
Cerebral palsy	17	2 794
Postnatal infections	4	2 688
Normal pre peri and postnatal history	17	3 437

probably explained by the true cumulative increase of the incidence of severe MR over the years of growth The term MR implies an ascertained diagnosis before the age of 16–18 years and it is a well known phenomenon that severely mentally retarded individuals show a progressive deterioration of test performance through the growing years (19 22) The true incidence of severe MR in our series was therefore considered to be representative of the children born 1959–64 aged 11–16 years and was 3 3 per 1 000 children alive at the age of one year

The number of deaths among the 122 mentally retarded children was 19 equally distributed over the two periods The median age at death was 3 years Only two children had died after 5 years of age The mortality rate thus amounted to 14% in the ages 1–5 years compared with 0 13% for the average population of the same ages in Sweden

As 16% of the mentally retarded children had died at the end of the follow up period (Dec 31 1975) the prevalence of severe MR at 11–16 years of age was calculated to be 2 8‰

Down s syndrome represented one third (39/122) of the total series and we thought it of interest to calculate its incidence per se As the mortality in Sweden for this syndrome during the first year of life is about 40% (12) the incidence was calculated on liveborns in the years concerned Table 2 shows that there was no significant difference between the two periods and that the calculated incidence figure of Down s syndrome per 1 000 live births was 1 46‰ This is quite in agreement with the findings in an earlier Swedish investigation (12) A lowering of the incidence to 1 32‰ during the years 1968–70 has been demonstrated in Sweden (15) Our figures do not contradict this decline which is probably due to the diminishing number of children born to women over 40 years of age Of the children with Down s syndrome only 3/39 (8%) died after their first year of life all between 1 and 5 years of age

The sex distribution

In the total series of 122 children 65 were boys and 57 girls giving a male excess ratio of 53 47 This sex difference is not significant

The children with Down s syndrome (39/122) showed a male excess ratio of 62 38 which is not statistically significant in this small series A significant male excess ratio has however been shown earlier in a larger Swedish series (15)

Birth weight

The mean birth weight in the total series was 3 173 g i e significantly ($p \leq 0$ 0001) lower than the mean (3 500) g in a normal Swedish population (13) 10 8% (14/122) had a low birth

Table 4 *The distribution of birth weight in relation to gestational age according to the Swedish standard curve in a series of 120 children with severe mental retardation*

	Observed frequency (%)	Expected frequency (%)
>+ S D	7 5	2 3
≤+2 S D >+1 S D	16 7	13 6
≤+1 S D >−1 S D	57 5	68 3
≤−1 S D >−2 S D	15 0	13 6
≤−2 S D	8 3	2 3

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≤−2 S D	8 3	2 3

Table 5 The frequency of epilepsy complicating mental retardation in different studies

Authors	County	Type of series	IQ	Ages	Epilepsy (%)
Penrose 1938 (17)	England	Institutional selected		All ages	28
Åkesson 1968 (20)	Sweden	Unselected	<−3 S D	All ages	21
Wallin 1974 (18)	Sweden	Unselected	<−3 S D	All ages	27
Eeg-Olofsson 1972 (5)	Sweden	Institutional selected	<70	0–15 years	32
Corbett & Harris 1974 (3)	England	Unselected	<50	0–14 years	32
Lindsjö 1974 (14)	Sweden	Unselected	<50	0–14 years	31
Present series 1977	Sweden	Unselected	<50	0–16 years	30

weight ≤2 500 g (LBW) compared to 4 3% in the average population and 39% in an unselected series of cerebral palsy (CP) children (10) during the same period There was none with a birth weight of 1 500 or less The difference in the proportion of LBW was highly significant ($p \leq 0\ 001$) in comparison with the normal as well as with the CP population No change through the years was observed

When grouping according to pathogenetic factors the mean birth weights were as shown in Table 3 It appears that the reduction of the mean birth weight was mainly referred to the children with a history of foetal deprivation of supply (7 11) with or without simultaneous histories of asphyxia/cerebral haemorrhage Children with CP as additional handicap to their severe MR consistently had a lowered mean birth weight Pathogenetically they *mainly represented one or both of the two* particular groups mentioned With the exception of Down s syndrome children with prenatal stigmata and a genetic or an untraceable aetiology had on an average normal birth weights The group with defined postnatal infections included one baby with a birth weight of 1 590 g appropriate for gestational age and in its second week of life a listeria meningitis followed by a severe hydrocephalic state The three other children in this group had birth weights of 2 620 2 760 3 780 g respectively

Gestational age

For 120/122 patients information on gestational age was obtained from obstetric records Thirty two out of 120 (27%) had been born at or before 37 completed weeks of gestation Fifteen out of 120 (12 5%) were born at or after 42 completed weeks Excluding the 39 children with Down s syndrmoe the figures were 21 and 15% respectively Compared to the general Swedish Birth Data Information Bank where the corresponding figures are 10 and 16% respectively the children with severe MR had a significantly ($p \leq 0\ 0001$) lowered gestational age

Birth weight in relation to gestational length

When comparing birth weights in relation to gestational ages according to the Swedish normal growth curve (6) the birth weights were not normally distributed There were too *many large and too many small for gestational* age (Table 4) the statistical calculations showing highly significant deviations ($p < 0\ 001$)

Associated CNS handicaps

Epilepsy (defined as recurrent epileptic seizures excluding convulsions during the first month of life and febrile convulsions under 5 years of age) was the most frequent associated CNS handicap and had occurred in 36/122 (30%) cases Twenty two *of 122 (18%) had* cerebral palsy in addition to their severe MR All syndromes of cerebral palsy were represented spastic/ataxic diplegia (9 cases) and dyskinetic syndromes (6 cases) most frequent

ly Ten of 122 (8%) of the children had epilepsy as well as cerebral palsy Severe hearing impairment was present in 4 cases and severe impairment of vision in 12 cases Seven children were hydrocephalic None had a concomitant spina bifida cystica Many of the patients had more than one of the above handicaps In total 51/122 (42%) had one or more additional CNS handicaps besides their severe MR

DISCUSSION

Most epidemiologic studies on severe MR have been restricted to prevalence data mainly employing the census method This has been well documented in the review of Abramowics & Richardson (1) who surveyed 27 community studies of severe MR from different parts of the world Very few researchers have coped with the incidence of severe MR McDonald s survey from Canada (17) in fact being the only one in recent years Compared with that study which gave an incidence of 4 6‰ for children alive at one year of age and born in 1958 in Quebec our investigation showed a lower incidence figure i e 3 3‰ Our estimated prevalence of 2 8‰ at 11–16 years was also lower than other age selected prevalence surveys An extremely thoroughly performed Swedish investigation with its census date in 1969 (19) revealed a prevalence of 4 6‰ at 10–19 years The number of cases in that series however is too small to make any statistical comparison with our result It is possible that the general high social welfare as well as the centralized and intensive perinatal care in Sweden during the last two decades might explain at least partly the lower incidence of severe MR compared with the majority of figures from other countries As will be demonstrated in part II of this study (8) we had only a few postnatal CNS infections and no case of hyperbilirubinaemia as the believed cause of severe MR together constituting only 3% of this series The corresponding estimated figure in McDonald s survey of children born in 1958 (17) was 11% indicating that in Sweden these two brain damaging factors among others had been minimized as causes of severe MR already before 1959 The higher prevalence figures in the ages 11–16 years well known from earlier series (1) are not merely due to differences in case finding procedures but also to a true cumulative increase of the incidence of severe MR (according to IQ definitions) through the years of growth As our series is considered to be complete the higher incidence figure during 1959–64 compared with the period 1965–70 is thought to be due to the latter explanation

The infant death rate (death during the first year of life) in Sweden has decreased progressively from 16 8‰ in 1959 to 11 0‰ in 1970 Furthermore perinatal gains in the form of fewer cerebral palsied children have been demonstrated (10) for the same period As will be shown in part II of this study (8) the pathogenetic factors were prenatal in 73% of the cases and perinatal in 10% The incidence of severe MR in the children born during the periods 1959–64 and 1965–70 was unchanged These data suggest that nowadays in Sweden cases of severe MR are only to a minor extent recruited from groups predominantly burdened with obvious perinatal and neonatal pathogenetic factors and that the decreasing infant mortality has not resulted in a higher incidence of severely mentally retarded children as some sceptics have feared

Our series of severe MR like some others (2 4) showed an increase in the proportion of pregnancies terminating at or before 37 completed weeks of gestation and resulting in LBW infants Åkesson (21) showed in 1966 a normal mean gestational length but more cases of unusually short as well as of unusually long gestations than expected His series however was limited to institutionalized cases and excluded all children with Down s syndrome and all those who had died before the census day Excluding the Down s syndrome children in our series the mean gestational age tended to

be normalized but was still significantly decreased ($p \leq 0.001$).

The association of very low birth weight with severe MR and cerebral palsy in earlier years has been well established (16). However among our LBW children there were no uncomplicated cases, appropriate for gestational age, and none weighing less than 1 500 g, indicating that with appropriate neonatal care routines the uncomplicated LBW infants were not at increased risk of acquiring severe MR. On the other hand, babies weighing more or less than 1 SD in relation to their gestational age were overrepresented in our series, i.e. the risk of acquiring severe MR is increased in those groups.

In the cerebral palsy study (11), the birth weights in relation to gestational ages were depressed with maintained variance, whereas in this study they diverged equilaterally, resulting both in more small for date and large for date babies.

The magnitude and importance of multihandicaps in severe MR are clearly demonstrated by our study, where 42% of the mentally retarded children had one or more CNS disabilities. This is in agreement with some other recent studies (14, 20). The most frequent problems in our series were connected with epilepsies, many of which were of a severe type, resistant to antiepileptic drugs and inviting to CNS-depressing multipharmacy. Our total figure of 30% is well in agreement with other reports (Table 5). The frequency of cerebral palsy has decreased during the period in question, also in MR children (10). In the present series, however, limited to severe MR, no decrease was found. 10 CP children were born in 1959–64 and 12 in 1965–70. CP was more than 100 times and epilepsy more than 30 times more frequent among the severely mentally retarded children than in the average Swedish population of the same ages. These high figures constitute an apparent illustration of the large body of neuropaediatric problems within the group of severely mentally retarded children.

ACKNOWLEDGEMENTS

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Submitted Sept 21 1976
Accepted Nov 4 1976

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CASE REPORT

INFANTILE POLYARTERITIS AND KAWASAKI DISEASE

A D SMITH

From the Department of Paediatrics Raigmore Hospital Inverness Scotland

ABSTRACT Smith A D (Department of Paediatrics Raigmore Hospital Inverness Scotland) Infantile Polyarteritis and Kawasaki Disease Acta Paediatr Scand 66 381 1977.—Polyarteritis in infancy is very rare difficult to diagnose and invariably fatal A 6-month-old girl who presented with a prolonged unexplained fever and was subsequently demonstrated at post mortem examination to have polyarteritis is described The combination of polyarteritis with some unusual presenting features suggests that the case described is one of the Mucocutaneous Lymph Node Syndrome (M L.N.S) or Kawasaki Disease Polyarteritis and Kawasaki Disease are discussed with reference to the case described

KEY WORDS Infantile polyarteritis Kawasaki Disease

Polyarteritis is uncommon in childhood is extremely rare in infancy and in the infant it presents as a unique form of this connective tissue disorder A number of cases have been reported from various countries in the last ten years (2 8 10) A further case in which the histopathological diagnosis of polyarteritis was made at post mortem examination is described and the implications of the findings are discussed

CASE REPORT

A 6-month-old girl became unwell 2 weeks before admission and was treated by her family doctor for otitis media and oral thrush with ampicillin and nystatin for 7 days The girl appeared to improve but her condition subsequently deteriorated and she was admitted to hospital with a fever vomiting and loose green stools neck stiffness and a rash The mother had also noticed a large gland in the infant s neck

On examination she was irritable cried constantly and held her neck retracted She had an erythematous rash on the trunk a large left cervical lymph node a left subconjunctival haemorrhage marked neck stiffness and a temperature of 40 2 C There was no evidence of oral thrush but the lips were dry and there was intense redness of both the lips and buccal mucosa The liver was enlarged and firm but there was no splenomegaly

The only abnormal results of the investigations on admission were a haemoglobin 9 9 g/dl white cell count 22 000×10⁹/l with 42% polymorphs 30% lymphocytes 2% eosinophils 8% monocytes 16% stab cells and 2% myelocytes a bilirubin of 40 micromol/l S G O T 175 I U S G P T 143 I U and alkaline phosphatase 171 I U Other investigations with normal results included microscopy and culture of cerebrospinal fluid blood and urine stool cultures and chest X ray The E S R was 28 mm in the first hour

She was treated with chloramphenicol penicillin and sulphadiazine in the expectation that the illness was due to a bacterial infection She remained ill with remittent fever and although her rash disappeared the neck stiffness persisted and the white cell count remained elevated above 25 000×10 /l She developed a moderate degree of abdominal distension and grunting respiration The liver function tests returned to normal with the bilirubin 9 micromol/l S G O T 46 I U S G P T 34 I U other investigations included IgG 12 00 g/l IgA 1 6 g/l IgM 0 88 g/l alpha foetoprotein and hepatitis B antigen not detected intravenous pyelogram and radiological and ultrasonic screening for an intra abdominal abscess normal and electron microscopy and tissue cultures for viruses were negative The Widal reaction and complement fixation titres to viruses and other agents including herpes simplex toxoplasma cytomegalovirus rubella and rickettsia were all less than 1 4

After 12 days the therapy was changed to gentamicin without improvement in her condition The erythematous

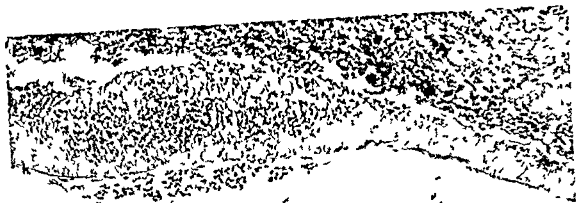


Fig 1 Major coronary artery—high power micrograph showing focus of arteritis with destruction of the elastic lamina and stenosis

Fig 2 Myocardium—half of the field shows a considerable loss of muscle cells and a heavy infiltrate of chronic inflammatory cells

rash reappeared and became florid and widespread She became severely pyrexial and died suddenly 17 days after admission

Post mortem results

The gross findings at post mortem examination consisted of a randomly distributed skin rash minor subarachnoid haemorrhage around the vertex and a smaller than normal thymus

The major microscopic abnormalities were present in the heart The main coronary arteries were affected by multifocal transmural destructive arteritis with severe luminal stenosis (Fig 1) There was multifocal necrosis of myocardium and most of the non necrotic tissue showed an active non specific myocarditis (Fig 2) Isolated examples of previous arteritic episodes were found in one lung and in relation to one lymph node The liver showed active non specific inflammation of many portal tracts This was associated with minor architectural disorganisation of the related limiting plates of lobule cells There was profound atrophy of the thymic cortex

Specimens of all tissues were processed for micro organismal culture (protozoa bacteria and viruses) with negative results The results of other post mortem investigations were antinuclear factor autoimmune fluorescence antibody tests and R A latex globulin test all negative serum IgE not detected and serum complement C_3 1 6 g/l

DISCUSSION

The term periarteritis nodosa was used in 1866 by Kussmaul and Maier to describe an inflammatory disease of medium and small arteries The more accurate term polyarteritis is now used The disease in adults is characterised by its diverse clinical manifestations and multi system involvement Classical infantile poly arteritis nodosa (I P N) is a diagnosis usually made only at post mortem examination but the clinical features and pathological findings distinguish this disease from that found in older children and adults

The sex incidence is equal and despite the variability of clinical manifestations a review of reported cases (8) suggested that a pattern of clinical features existed Later reports support this view (2 10) The features are a prolonged fever unresponsive to antibiotic therapy an erythematous rash an upper respiratory tract infection with conjunctivitis and a persistent leukocytosis in the absence of infection These authors suggest that these features in association with urinary protein or cells cardiomegaly or changes indicating left ventricular hypertrophy or ischaemia on the E C G should alert the clinician to the diagnosis of I P N Other findings include diarrhoea peripheral oedema neck stiffness convulsions and hemiplegia The diagnosis however remains difficult and is not usually clarified by biopsies The disease is usually fatal and death may be sudden and unexpected often at an early stage in the illness

Pathological changes are usually restricted to the major coronary arteries which are invariably and severely affected by polyarteritis Aneurysmal dilatation is often present and has been demonstrated during the illness by cardiac cine angiography (1) Death results from coronary thrombosis or rupture of an aneurysm

The case described demonstrates many of the features of classical I P N However three prominent clinical signs of this case are not usually described in I P N and require further consideration These are the cervical lymph adenopathy the intense redness of the lips and buccal mucosa and the conjunctival haemorrhage

In 1974 Kawasaki et al (5) reviewed an acute febrile disease which Kawasaki had originally described in 1967 in Japan (4) and which affected infants and young children The main features are prolonged fever a polymorphous rash redness of the lips tongue and mouth conjunctival congestion redness and oedema of the extremeties with membranous desquamation of the fingertips in the convalescent stage and acute swelling of the cervical lymph nodes They called this the Mucocutaneous Lymph Node Syndrome (M L N S) and it has now been designated Kawasaki Disease (K D) Other features include cardiac failure diarrhoea urinary protein or cells leukocytosis mild anaemia an increased E S R aseptic meningitis and raised serum transaminases The incidence which seems to be increasing is highest in the first year of life and greater in males than in females (1 5 1) All the original cases (4) recovered



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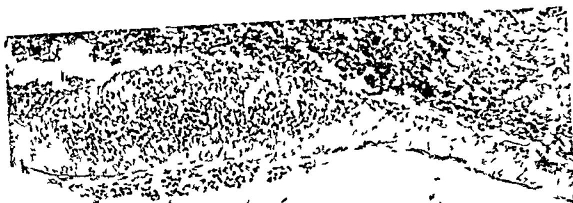


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The aetiology is unknown and although a hypersensitivity mechanism is implicated in the adult form of polyarteritis, evidence of this is usually lacking in infants Raised serum IgE has, however been described in both I P N (6) and K D (7) The role of any micro organism in the pathogenesis of the disease has not yet been confirmed There is no satis factory treatment for either I P N or for the usually self limiting condition of K D

Kawasaki Disease accounts for the features of the case reported, it is submitted that mem branous desquamation of the fingertips and coronary artery aneurysms were not seen be cause of the early death of the infant The re lationship between I P N and K D is unclear at present but I P N may represent the fatal end of a disease spectrum which is more com monly manifested as the relatively benign K D If this is proved to be true then a solution to the aetiology of arterial inflammatory dis ease may be nearer The increasing incidence of K D may be the result of an environmental factor yet to be identified (7)

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CASE REPORT

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ABSTRACT Friedli B Renevey F and Rouge J C (Clinique Universitaire de Pediatrie Geneva Switzerland) Complete heart block in a young child presumably due to Mycoplasma Pneumoniae Myocarditis Acta Paediatr Scand 66 385 1977.—The case is described of an 18 months old boy with sudden onset complete heart block heralded by Stokes-Adams attacks General signs of viral illness preceded and accompanied the syndrome this along with angiographic evidence of a poorly contracting left ventricle led to the diagnosis of non bacterial myocarditis Serologic tests disclosed a significant rise in antibodies against mycoplasma pneumoniae (1/16 to 1/128) The His-bundle electrogram showed a block above the His-bundle but fairly widespread damage to the conduction system is suspected The complete heart block proved to be permanent and a fixed rate pacemaker had to be implanted

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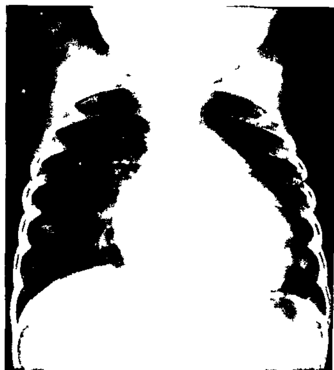


Fig 1 Chest X ray on the day of admission showing *cardiomegaly and slight pulmonary venous congestion*

catheterization was performed including a His bundle electrogram The hemodynamic data were normal apart from an increased left ventricular end-diastolic pressure (12 mmHg) A left ventricular angiogram showed enlargement of the cavity and poor left ventricular contractility The His bundle potential was found with some difficulty it was recorded as a rather slow deflection preceding every V wave thus locating the CHB above the His bundle (Fig 3) The H–V interval was 25 msec which is *within normal limits for this age group (1) A transvenous* pacing electrode was left in the right ventricle and the child was digitalized Sixteen days after the initial Stokes Adams attacks there was no sign of return to sinus rhythm and a permanent epicardial pacemaker at a fixed rate of 95/min was implanted

Among a variety of complement fixation tests done (Table 1) the titre of antibodies against mycoplasma

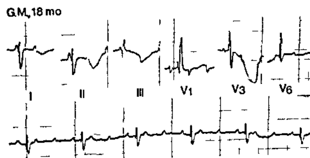


Fig 2 ECG on admission showing complete heart block a wide QRS complex with *RBBB pattern* and T wave abnormalities

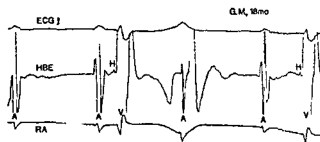


Fig 3 His bundle electrogram The upper trace is a surface ECG lead I (ECG I) in the middle is the His bundle electrogram (HBE) the lower trace is a recording from the right atrium (RA) Note rather sluggish H-deflection preceding the V waves

pneumoniae rose significantly from 1/16 on the day of admission to 1/128 ten days later A high titre of antibodies against Respiratory Syncitial Virus on admission fell subsequently *probably indicating infection with this agent* prior to the myocarditis in relation with the upper respiratory infection three weeks before admission Cold agglutinins were looked for late in the course of the illness and were not found

The child left hospital a week following implantation of the pacemaker and has since been asymptomatic Digoxin was discontinued after one month

At follow up 20 months after the Stokes Adams attack the child is very well requires no medication and is de*veloping normally There has been no sign of return to* normal sinus rhythm

COMMENT

CHB in infants and young children is usually of congenital origin Death from congestive heart failure may occur in this condition during early infancy most patients surviving to the second year of life are likely to be asymptomatic throughout childhood Stokes Adams attacks occurring in a small percentage only

Table 1 *Results of complement fixation tests*

	Admission	10 days after
Q Fever	Neg	Neg
Psittacosis	Neg	Neg
Mycoplasma pneumoniae	1 16	1 128
Influenza A	Neg	Neg
Influenza B	1 8	1 8
Respiratory syncitial virus	1 128	1 64
Adenovirus	Neg	Neg
Mumps	1 8	1 8
Enterovirus	Neg	Neg

(6) In this age group acquired heart block un related to congenital heart disease is rare in the large series published by Nakamura & Nadas (7) only 4 children out of 61 with CHB fell into this category One had heart block with endocardial fibroelastosis in one the origin was unknown and in two myocar ditis was strongly suspected In the case pres ented here normal heart rates had been re corded by the pediatrician on several occa sions prior to the current illness Bradycardia was first noted following two Stokes Adams attacks There is therefore little doubt that the A–V block was acquired Considering the etio logy we believe that there is much evidence in favour of infectious non bacterial myo carditis the child had a febrile illness with upper respiratory tract infection 20 days and again 3 days before onset of the Stokes Adams attacks he was febrile on admission had a red throat and a slight leucocytosis with 15% mononuclear cells suggesting viral infection There was evidence of diffuse myocardial dis ease with a very large heart pulmonary ven ous congestion and a poorly contracting left ventricle on the cine angiogram as well as a high left ventricular end-diastolic pressure

Among the many non bacterial agents proven or suspected to cause myocarditis mycoplasma pneumoniae has been in frequently reported (3 5) In the present case this organism is suspected because of a signifi cant rise of titre of mycoplasma pneumoniae antibodies Pneumonia was not present on ad mission as it was in the case of El Khatib et al (3) however pneumonia is not a constant feature of mycoplasma pneumoniae infection it was absent in three of twenty cases collected by Lambert (5)

CHB due to viral myocarditis has been in frequently reported In one case mumps virus was strongly suspected as being the cause (4) in all others the exact etiology remained ob scure In almost all cases reported to date the outcome was benign with return to normal sinus rhythm within a few days this unfor tunately did not happen in the present case and a fixed rate permanent pacemaker had to be implanted two weeks after the initial episode of block We know only of one case of ac quired heart block requiring permanent pacing in a young child reported by Serradimigni et al (9) the etiology in that case remained un known although a viral origin was suspected

On the basis of the clinical history (presence of Stokes Adams attacks) and particularly the surface ECG we had assumed that our patient had CHB distal to the His bundle Indeed the ventricular rate was slow and the QRS com plexes wide However the His bundle electro gram revealed a block proximal to the His For the following reasons we believe that damage to the conduction system is diffuse

1) the His bundle electrogram suggests damage to the A–V nodal area (CHB above His)

2) the ECG indicates damage to the right bundle branch (complete RBBB)

3) a lesion in the junctional area and bundle of His must be suspected to explain the rather slow junctional escape rhythm which could not be accelerated by Isoproterenol or Atro pine as well as the occurrence of Stokes Adams attacks The His bundle electrogram further substanciates this hypothesis Indeed according to Puech (8) a sluggish H-deflec tion as seen in our case indicates damage within the His bundle

Interestingly right bundle branch block was found in two previously reported cases (9 2) it has persisted in the latter case after return to sinus rhythm

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Submitted June 11 1976
Accepted Dec 10 1976

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Acta Pædiatr Scand 66 389–392 1977

CASE REPORT

INFANTILE CONVULSIONS
TRANSIENT INFANTILE HYPOCALCEMIA ASSOCIATED WITH SKELETAL DEFORMITIES

J L VAN DER HORST and J F M HÖLSCHER

From the Department of Paediatrics Sint Lucas Ziekenhuis Amsterdam The Netherlands

ABSTRACT Van der Horst J L and Holscher J F M (Department of Paediatrics Sint Lucas Ziekenhuis Amsterdam The Netherlands) Infantile convulsions Transient infantile hypocalcemia associated with skeletal deformities Acta Paediatr Scand 66 389 1977.—An infant with skeletal deformities and hypocalcemic convulsions is described The convulsions which were first noted in the 5th week of life as well as the low serum calcium level were transient The skeletal deformities consisted of deformation of the terminal phalanges of the right third finger and right second toe syndactyly of the third and fourth finger of both hands and left pes equinovarus X ray examination revealed abnormal shape and structure of some bones of the hands The most likely diagnosis is transient congenital idiopathic hypoparathyroidism which has not yet been described in association with congenital skeletal anomalies

KEY WORDS Infantile hypocalcemia tetanic convulsions transient congenital idiopathic hypoparathyroidism multiple congenital skeletal anomalies

The paediatrician confronted with a convulsive infant will undoubtedly consider metabolic disorders as an etiologic factor When the calcium concentration in the blood of such an infant is sufficiently decreased the diagnosis is hypocalcemic tetany Hypocalcemic tetanic convulsions often resemble epileptic seizures especially in the neonate

Serum calcium is controlled by the parathyroid glands Moreover normal renal function—especially phosphate excretion—is necessary for maintaining calcium homeostasis Also intestinal calcium and phosphate absorption should be unimpaired (4) In tetany one of these factors is not properly functioning

CASE HISTORY

The patient Mustafa a Turkish infant was admitted to the hospital at the age of 4 days In the first trimester of pregnancy the mother tried without result to induce an abortion by jumping from a great height Otherwise pregnancy and delivery were uneventful and birth weight was in the normal range Skeletal deformities were unknown in both parental families

Ten days before admission the parents recognized a convulsion the child was cyanotic displayed seizures and foamed at the mouth On the day of admission several convulsions occurred and the child was admitted while having a convulsion This was successfully treated by intravenous diazepam Mustafa remained restless with small myoclonic jerks

Physical examination

The face was round (Fig 1) The terminal phalanges of the right third finger and the right second toe were deformed (Figs 2 and 3) There was syndactyly of the third and fourth fingers of both hands (Fig 2) The left foot was a pes equinovarus (Fig 4) while in the right leg a constricting band was visible

X rays showed absence of the terminal phalanges of the third and fourth finger of the right hand (Fig 5) a broad and curved first metacarpal bone and short broadened phalanges with abnormal bone structure

Biochemical data and clinical course

Blood glucose and blood urea were in the normal range respectively 5 2 mmol/l and 1 3 mmol/l Aspect and composition of the stools were normal

Serum calcium was low 1 6 mmol/l Serum phosphate

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Fig 1 Patient Mustafa at admission

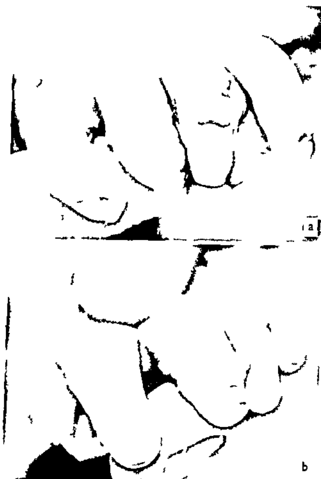


Fig 2 Right and left hand with deformity of right third finger and bilateral syndactyly of fingers

was 2 0 mmol/l and alkaline phosphatase was 1 28 nmol s⁻¹/l Serum magnesium was not determined

Daily intramuscular administration (2) of 10 20 U S P units of parathyroid extract (Parathormon Lilly) for 12 days resulted in a rise of serum calcium to 2 2 mmol/l Subsequently serum calcium fell to 1 6 mmol/l on the 8th hospital day in spite of continued administration of PTE (Fig 6) Phosphate intake was not excessive initially the child was fed on human milk and later on a mixture of human and humanized milk (Almiron M2) with a normal Ca/P ratio

With anticonvulsive therapy consisting of diazepam and phenobarbital convulsions no longer occurred but the patient remained restless and irritable for several days after admission

Administration of PTE was stopped 13 days after admission Still serum calcium rose spontaneously to a level of 2 1 mmol/l 10 days later (Fig 6) Four days later administration of PTE was resumed this time in a dosage of 60 U S P units Serum calcium continued to rise and reached a normal value (2 4 mmol/l) after another 5 days At that time serum phosphate was 1 7 mmol/l Serum calcium remained normal after cessation of PTE treatment 4 days later (Fig 6)

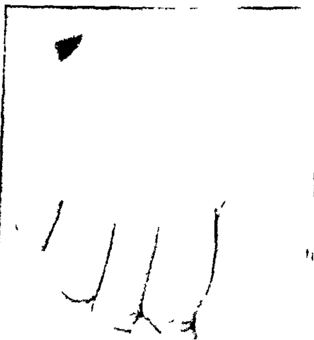


Fig 3 Right foot with deformity of second toe

DISCUSSION

The patient described had hypocalcemic tetany Renal insufficiency excessive intake



Fig 4 Pes equinovarus of left foot constricting band in right leg



Fig 5 X ray of hands absence of terminal phalanges fingers III and IV of the right hand

of phosphate and intestinal malabsorption (4) could be excluded as causes for the lowered serum calcium level

Neonatal hypocalcemia was unlikely since the first symptoms appeared in the 5th week of life *Rickets* (5) was excluded by the normal concentration of alkaline phosphatase and the absence of rachitic changes on the X ray photographs

A further possibility was *hypoparathyroidism* The *chronic* type of this disease could be ruled out since hypocalcemia did not persist

Transient hypoparathyroidism can be due to *maternal hyperparathyroidism* (6) The mother of the child did not show any signs of this condition however the serum calcium was 2 4 mmol/l phosphate 0 9 mmol/l and alkaline phosphatase 0 20 nmol s^{-1}/l

Fig 6 Biochemical data of the patient upon admission to hospital



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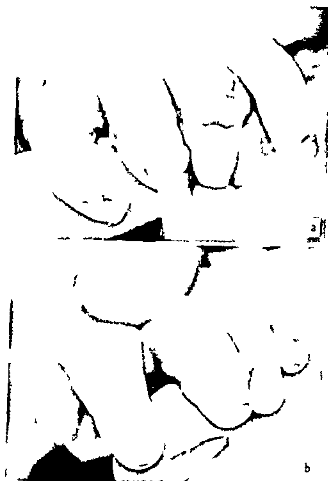


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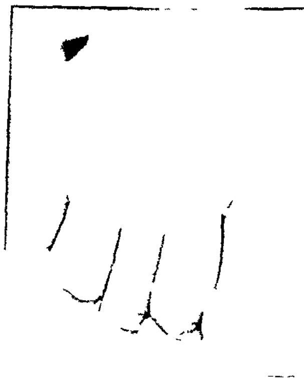


Fig 3 Right foot with deformity of second toe

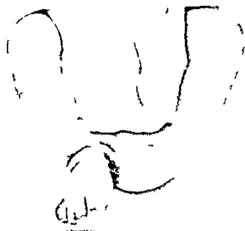


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Fig 6 Biochemical data of the patient upon admission to hospital

Finally temporary hypocalcemia is seen in *transient congenital idiopathic hypoparathyroidism* described by Fanconi & Prader (3) and also by Balsan & Alizon (1) It has been suggested (3) that the cause of this condition is a primary congenital hypoplasia or dysplasia of the parathyroid glands which will become hyperplastic to correct the hypocalcemia The infants described by these authors became hypocalcemic in the first weeks of life as our patient

The positive response of serum calcium to administration of PTE was regarded by Fanconi & Prader as proof of the presence of hypoparathyroidism In our case the dose of PTE administered was much lower however and therefore the diagnosis was not definitely established Still the symptoms clinical course and spontaneous recovery are in agreement with transient congenital idiopathic hypoparathyroidism

The further course of Mustafa s illness was favourable convulsions did not occur any more Just as in the patients of Fanconi & Prader the serum calcium level remained normal When this paper was written Mustafa was 34 months old Psychomotor development as well as growth in stature had been satisfactory

To conclude although not proved Mustafa s illness should be diagnosed in our opinion as transient congenital idiopathic hypoparathyroidism The association fo this condition with multiple congenital skeletal anomalies has not been reported before

ACKNOWLEDGEMENT

We wish to thank Dr R Steendijk reader Department of Paediatrics University of Amsterdam for his valuable advice in preparing this paper

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Submitted May 25 1976
Accepted Oct 29 1976

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Acta Pædiatr Scand 66 393–395 1977

CASE REPORT

DISSEMINATED ARTHRITIS AND OSTEITIS BY CANDIDA ALBICANS IN A TWO MONTH OLD INFANT RECEIVING PARENTERAL NUTRITION

L BUSINCO G IANNACCONE D DEL PRINCIPE S LUCARELLI
E CARDI and E REZZA

From the Department of Paediatrics University of Rome Rome Italy

ABSTRACT Businco L Iannaccone G Del Principe D Lucarelli S Cardi E and Rezza E (Department of Paediatrics University of Rome Italy) Disseminated arthritis and osteitis by Candida albicans in a two month old infant receiving parenteral nutrition Acta Paediatr Scand 66 393 1977 —The case of a two-month-old female infant who after a severe diarrhoea treated with prolonged intravenous infusion in peripheral veins alternated with total parenteral feeding developed a Candida albicans septicemia (accompanied by disseminated intravascular coagulation syndrome) is reported The course of her disease was also complicated by multiple foci of osteoarthritis in both knees in the left hip and in several long bones Radiographically the foci of Candida osteitis appeared as fine erosion of the cortex and minute round areas of osteolysis in the spongiosa surrounded by a rim of perifocal sclerosis During the acute stage of Candida sepsis a transitory cellular immunodeficiency was present Treatment of Candida infection by 5 fluorocytosine was followed by complete recovery

KEY WORDS Osteoarthritis Candida albicans parenteral nutrition

Through the recently developed method of parenteral nutrition it is now possible to save infants who in the past died of starvation Nevertheless in patients so treated a conspicuous number of complications has been reported such as bacterial and mycotic sepsis thromboflebitis bone lesions hyperosmolar dehydration hypokalemia and hypocalcemia (4 6 7) Candida albicans osteoarthritis a complication previously practically unknown has been reported on three occasions in infants in the last four years (1 8 9) This is the case report of a two month old female infant who developed a sepsis due to Candida albicans with disseminated osteomyelitis during parenteral feeding and was successfully treated with 5 fluorocytosine

CASE REPORT

The patient was admitted to the hospital on December 30 1973 at 16 days of age because of high fever and severe diarrhoea Three days before her twin sister had died of a brief and violent episode of diarrhoea

She was born following normal twin pregnancy and delivery Her birth weigth was 2450 g The neonatal period was uneventful She was fed artificially with dried milk At admittance she was markedly dehydrated but on physical examination no other abnormalities were detected

Laboratory investigations gave the following results Hematocrit 73% hemoglobin 19 g/100 ml white blood cells 13 400/mm³ (4% neutrophils 6 eosinophils 2 monocytes and 50 lymphocytes) base-excess 9 mEq/l B U N 19 mg/100 ml Culture from a rectal swab yielded few colonies of *Staphylococcus aureus* and abundant growth of *Klebsiella aerogenes* Widal Wright test was negative three weeks after the admission

During the first month the diarrhoea persisted unabated Treatment consisted of infusions in peripheral veins of glucose saline solutions alternated with periods of

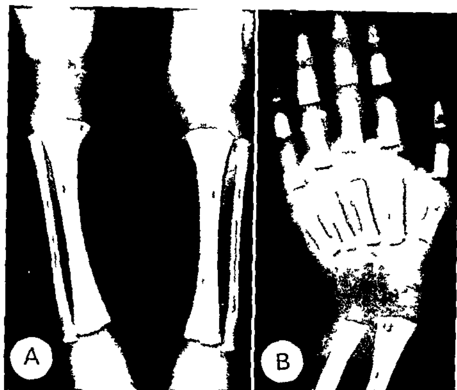


Fig 1 (A) Both knees especially the left one are swollen Small osteolytic areas are visible in the femoral and tibial metaphyses and in both anklebones (B) A rounded area of osteolysis sharply de lineated by perifocal sclerosis is evident in the distal metaphysis of the right radius

parenteral feeding with a solution containing per 100 ml (g for Aminosol and glucose mEq for the electrolytes) Aminosol 2 6 glucose 15 Na 5 K 2 Mg 1 2 Ca 1 3 P 2 Cl 6 and methicillin 100 mg Other antibiotics including gentamycin and cephalosporin were also provided

After one month of treatment with subsequent moderate improvement of the child general conditions and diarrhoea a sudden onset of large ecchymotic areas shock coma and generalised clonic seizures occurred The presence of thrombocytopenia prolonged prothrombin time and PTT along with a highly positive protamine sulphate test accompanied by deficiency of factor II V VII VIII IX led to a diagnosis of disseminated intravascular coagulation syndrome Treatment was given *for five days (heparin 200 U/kg every 4 hours hydrocorti*sone 25 mg/kg every 8 hours) After 13 days while the ecchymotic areas were disappearing a new set of complications took place fever and oral thrush appeared and Candida albicans was found in blood culture In the following days the knees became swollen and painful the left thigh being kept in a flexed position The ankles and the shoulder joints were subsequently involved On radiological examination there were signs of articular effusion in both knees with widening of the articular spaces and capsular distension The metaphyses of several long bones and of the tarsal bones showed fine erosions of the cortex and several minute areas of osteolysis in the spongiosa round shaped and sharply delineated by perifocal sclerosis (Fig 1) Similar lesions were present in the nuclei of both anklebones Neither lesions of the diaphyses nor periosteal reaction were observed

Candida albicans was isolated repeatedly from the whitish purulent material obtained by arthrocentesis of both knees and from blood cultures

Treatment with 5 fluorocytosine (Ancotil®) was immediately begun 100 mg/kg were administered daily intravenously in three divided doses After 22 days of i v treatment which was well tolerated the drug was administered by mouth at the same daily dose for a further period of 6 weeks Cultures from knee exudates and blood were negative after 13 and 20 days respectively Osteoarthritis of the left hip produced a dislocation of the femural head which was treated by means of traction on the lower limb and surgical reduction

The patient was discharged at five months of age in good health At a follow up 14 months later there was complete disappearance of the bone lesions

METHODS

Immunological tests were performed as described in our previous papers (5 10)

System B evaluation

Serum immunoglobulins levels were IgG 730 IgM 25 IgA 31 mg/100 ml during the acute stage of infection following recovery and at later follow up all values were increased Anti A and anti B isohaemoagglutinins were present

Three doses of typhoid parathyphoid vaccine caused a normal antibody response The Candida agglutinins were 1 40 during the acute stage and absent after 4 months The number of peripheral lymphocytes with membrane immunoglobulins and of those with receptors for C_3 (B lymphocytes) were normal

System T evaluation (Table I)

Delayed skin test sensitivity to Candida (Dermatophytin 1 10 Hollister Stier) PPD (10 UT Sclavo) PHA (μg 2 Wellcome) and DNCB were all negative initially but during convalescence Candida and DNCB responses became

Table 1 *Immunological evaluation during Candida septicemia and after the recovery*
Normal values in parentheses

	During Candida septicemia	After recovery 70 days	200 days
Candida antibodies (<1 14)	1 40	1 20	0
Delayed skin test			
Candida	Neg	+++	+++
PHA PPD SK	Neg	Neg	Neg
DNCB	Neg	Neg	+++
Rosettes forming lymphocytes (61±5)	2?	67	65
Response of lymphocytes to PHA stimulation index (>10)	9	34	30
In vitro lymphocyte response to Candida stimulation index (>10)	?	10	14

positive The proportion of circulating T lymphocytes evaluated by E rosette test was diminished In vitro responses of lymphocytes to PHA and to Candida were weak during the acute stage of Candida sepsis but became normal at recovery

The nitro-blue tetrazolium reduction of neutrophils was normal

DISCUSSION

Recently sepsis by Candida albicans has been reported in patients treated by prolonged parenteral nutrition by means of a central venous catheter (2 4 6) In our patient repeated intravenous infusions into peripheral veins were administered for prolonged periods but other factors favouring Candida infection were also present diarrhoea undernutrition treatment with antibiotics and corticosteroids Some of these factors are probably responsible for the transitory cellular immunodeficiency observed during the acute phase of the disease Moreover it has been suggested (6) that the hypertonic solution of glucose commonly used may predispose to Candida infection and it has been recently observed in man that hyperglycemia depresses the phagocytic properties of neutrophils (3)

Multiple foci of osteolysis were elicited in this infant X ray findings revealed the presence of small areas of radiolucency in the cortex at the metaphyseal level surrounded by a slight sclerotic reaction This peculiar radiological picture appears to be the hallmark of Candida osteomyelitis as it also was described in the few cases reported up to now (1 8 9) The localisation in the tarsal bones observed in our patient has never been reported

The effectiveness of 5 fluorocytosine was confirmed and also noteworthy appears the good tolerability of the drug which was administered for 9 weeks

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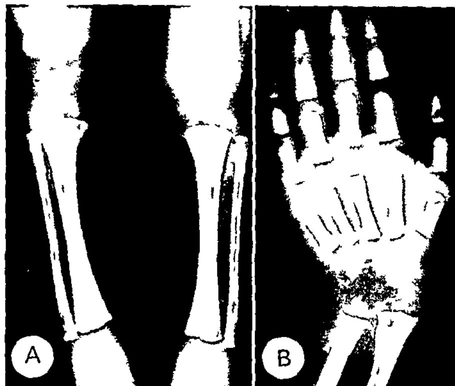


Fig 1 (A) Both knees especially the left one are swollen Small osteolytic areas are visible in the femoral and tibial metaphyses an in both anklebones (B) A rounde area of osteolysis sharply de lineated by perifocal sclerosis is evident in the distal metaphysis o the right radius

parenteral feeding with a solution containing per 100 ml (g for Aminosol and glucose mEq for the electrolytes) Aminosol 2 6 glucose 15 Na 5 K 2 Mg 1 2 Ca 1 3 P 2 Cl 6 and methicillin 100 mg Other antibiotics including gentamycin and cephalosporin were also provided

After one month of treatment with subsequent moderate improvement of the child general conditions and diarrhoea a sudden onset of large ecchymotic areas shock coma and generalised clonic seizures occurred The presence of thrombocytopenia prolonged prothrombin time and PTT along with a highly positive protamine sulphate test accompanied by deficiency of factor II V VII VIII IX led to a diagnosis of disseminated intravascular coagulation syndrome Treatment was given for five days (heparin 200 U/kg every 4 hours hydrocortisone 25 mg/kg every 8 hours) After 13 days while the ecchymotic areas were disappearing a new set of complications took place fever and oral thrush appeared and Candida albicans was found in blood culture In the following days the knees became swollen and painful the left thigh being kept in a flexed position The ankles and the shoulder joints were subsequently involved On radiological examination there were signs of articular effusion in both knees with widening of the articular spaces and capsular distension The metaphyses of several long bones and of the tarsal bones showed fine erosions of the cortex and several minute areas of osteolysis in the spongiosa round shaped and sharply delineated by perifocal sclerosis (Fig 1) Similar lesions were present in the nuclei of both anklebones Neither lesions of the diaphyses nor periosteal reaction were observed

Candida albicans was isolated repeatedly from the whitish purulent material obtained by arthrocentesis of both knees and from blood cultures

Treatment with 5 fluorocytosine (Ancotil®) was immediately begun 100 mg/kg were administered daily intravenously in three divided doses After 2? days of i v treatment which was well tolerated the drug wa administered by mouth at the same daily dose for a furthe period of 6 weeks Cultures from knee exudates and bloo were negative after 13 and 20 days respectively Osteoar thritis of the left hip produced a dislocation of the femura head which was treated by means of traction on the lowe limb and surgical reduction

The patient was discharged at five months of age i good health At a follow up 14 months later there wa complete disappearance of the bone lesions

METHODS

Immunological tests were performed as described in ou previous papers (5 10)

System B evaluation

Serum immunoglobulins levels were IgG 730 IgM 25 IgA 31 mg/100 ml during the acute stage of infection following recovery and at later follow-up all values were increased Anti A and anti B isohaemoagglutinins were present

Three doses of typhoid parathyphoid vaccine caused a normal antibody response The Candida agglutinins were 1 40 during the acute stage and absent after 4 months The number of peripheral lymphocytes with membrane immunoglobulins and of those with receptors for C_3 (B lymphocytes) were normal

System T evaluation (Table I)

Delayed skin test sensitivity to Candida (Dermatophytin 1 10 Hollister Stier) PPD (10 UT Sclavo) PHA (µg 2 Wellcome) and DNCB were all negative initially but during convalescence Candida and DNCB responses became

Acta Pædiatr Scand 66 397–402 1977

CASE REPORT

TRISOMY 8 SYNDROME

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ABSTRACT Bernsen A H Rasmussen K and Nielsen J (Institute of Psychiatric Demography and Cytogenetic Laboratory Psychiatric Hospital Risskov Denmark) Trisomy 8 Syndrome Acta Paediatr Scand 66 397 1977.—A case report of trisomy 8 in a 17 year-old mentally retarded female is presented and the previously reported cases of trisomy 8 are reviewed in an attempt to establish the clinical characteristics of the trisomy 8 syndrome

KEY WORDS Autosomal chromosome aberration trisomy 8 mental retardation

Before 1970 there was no possibility of distinguishing between the chromosomes in group C but since 1970 (5) a number of different banding techniques have made it possible to identify each chromosome The importance of this is evident in genetic counselling in order to prevent mental retardation due to chromosome abnormalities One of the mental retardation syndromes is trisomy 8 first described in 1971 by de Grouchy et al (13) The present report deals with a case of trisomy 8 identified by chromosome examination as part of an epidemiological survey on severely mentally retarded children in the community of Århus Denmark (3) and it includes a review of previously presented cases of trisomy 8

CASE HISTORY

The patient (B S) is a 17 year old female the second child of parents who were both ?9 years old when she was born There is no consanguinity and the mother has had no miscarriages or stillbirths In the third month of the mother s pregnancy the 4 year older sister had rubella but the mother was unaffected During the whole pregnancy the mother was treated with 5 mg del ortin daily on account of allergic rhinitis and had X ray examinations of thorax and nasal sinuses Full term delivery at home without complications or signs of asfyxia birthweight 3 000 g length 51 cm She was breastfed till the age of 5 months At ten months old she was referred to hospital for suspected meningitis which however was not confirmed examination showed that the was not able to sit without support and had sideropenic anemia Hb was 5 7 mmol/l X rays of the lungs heart cranium and lower extremities were normal

At the age of 18 months she was referred to the paediatric department and adenoid vegetations were removed as she had had several attacks of catarrhal fever and otitis media The mother stated that she could sit alone 12 months old and walk at 17 months At the examination she behaved like a 6 month old child smiled fixated and grasped things with both hands sat without support but was unable to walk or stand unsupported She did not play only uttered some unintelligible sounds and showed no interest in other persons Facial appearance coarse features with a large mouth flat glabella and a slightly prominent forehead she was oligomimic and hypotonic The skin was in general dry and rough Extremities were long and slender with long fingers and toes the skin over the kn e and elbow joint was tense but without contractures Cardiac examination and EEG were normal WR and toxoplasmosis titers were negative No further examinations were performed to establish the etiology of the mental retardation

No exact information concerning her behaviour for the next 2–3 years is available but she seems to have been difficult to manage hyperactive destructive jealous with temper tantrums sleep-disturbances and only slow mental development

She attended a normal kindergarten from the age of 4 years which altered her behaviour so that she became

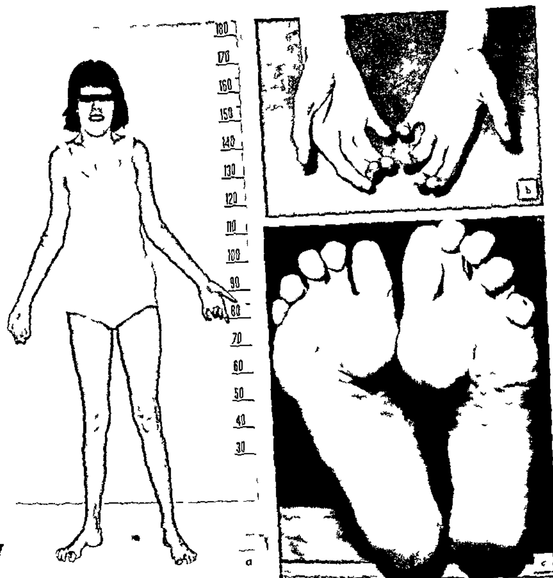


Fig 1 (*a b c*) The proband at the age of 17

19 21 25 29) and 9 cases of *partial trisomy* 8 (12 20 24 26 30)

Atkins et al (2) found it remarkable that about 30 individuals with this form of trisomy have been described in the short period from 1971 to 1974 which indicates that the condition is not extremely infrequent The incidence at birth is however unknown Only one case of C trisomy probably *trisomy 8* was found among 54 749 newborn *children* examined in USA Canada USSR Great Britain and Denmark This indicates that *the* frequency is comparatively rare probably 1 per 25 000–50 000 children

Taking into consideration that three quarters *of the* reported cases concerned partial *trisomy 8* or trisomy 8 mosaicism of varying *degree* which could account for the variations in the clinical manifestations we found it interesting to tabulate the different parameters described to each of three subtypes *full* mosaic and partial trisomy 8 (Table 1)

The picture became rather confusing *how*ever and showed no tendency towards

calmer and less destructive She was now able to walk and run with somewhat uncontrolled movements could manage eating was enuretic could say only a few simple words and understand very simple instructions

At the age of 5 6 years the speech therapist referred her to the mental retardation service but she continued in the normal kindergarten She was now clean and dry could help dressing herself and do small tasks under supervision She could talk in short sentences but the impressive language was better than the expressive

She was still somewhat aggressive and tyrannized her mother but showed more interest in both children and adults

At well over 7 years she started in the school for mentally retarded where she was difficult to manage hyperactive easily provoked by small alterations in the daily routine She had some routines of her own and tended to perseverate Her speech showed improvement but with very immature grammar and altered articulation She remained in the lower grades i e preschool classes until August 1970 when she was 12 when 3 hours of formal teaching were instituted From August 1971 she received formal teaching for 6 hours daily She is making slow progress the main difficulties being lack of concentration speech retardation and pronounced infantile behaviour

She still lives at home constant supervision is necessary in dressing and personal hygiene She cannot accept any personal responsibility and occupation is difficult she demands a great deal of attention and her sleep is restless Social development does not exceed 4 years at the age of 17 years

Psychological examinations

1964 6 5 years old) Binet Simon IQ=44
1967 (8 7 years old) Leiter IQ 55+5=60
1970 (11 8 years old) Binet Simon IQ=56 Leiter 51+5
1973 (15 0 years old) PPV test WISC CAT and Rorschach

The general impression of the first three examinations is good sense of orientation form and colour perception primitive language and indistinct pronunciation The last examination shows that she presents a slight gross motor incapacity talks a lot quite fluently but with impaired fonology she seems comfortable in the test situation but contact is superficial The cognitive tests show a mentally retarded female at the debile–imbecile level her form–colour perception facilitates simple puzzles while she fails in tests requiring reasoning

The personality tests reveal a rather harmonious girl whose main difficulty is that she cannot stop being naughty

Present state (16 years 11 months Fig 1*a b c*)
The general condition and nutritional state is good There is protruding forehead the area over the anterior fontanelle is large and hollow The hair limits are low frontally and occipitally The ears are normally differentiated and placed There is slight hypertelorism but neither strabismus nor epicanthus The nose is normal She has a large mouth with a high arched palate and protruding alveolar processes but no cleft palate and a normal tongue The neck and thorax are normal with scanty breast development (menstruation has not yet occurred) The hips are slender and axillary and pubic hair growth is normal She has long slender arms with cubitus valgus 5 extension defect in both elbows and a slight flexion defect long slender fingers with flexion contractures on the 4 ulnar fingers Mobility of the thumbs is normal The legs and feet are long slender with normal movements of the hips knees and ankles Alle toes show flexion contractures especially on the right foot The feet show cavus tendency and have hammer toes Deep skin furrows are found in the first and second interdigital spaces on the soles

Chromosome examination

Chromosome examination (Fig 2) was made on 48 hours lymphocyte cultures Q-banding and R banding were obtained with the Quinacrine–Mustard method (5) and the BUDR Acridin Orange method (10) 40 cells were analysed all of them contained 3 of the chromosome number 8 Banding patterns revealed the supernumerary chromosome number 8 to be deleted with breaking point at p 12 Thus the karyotype was 47,XX+del (8) (p 1?) Both parents and the elder sister had normal karyotypes

Dermatoglyphic investigation

The finger print pattern and the total finger ridge count (81) agree with previous findings that in trisomy 8 arches are increased in frequency and the total finger ridge count tends to be lower than that in English controls (♀126 97 ±1 82 (16))

On the palms the pattern configurations differ from those found in eleven other cases but show features typical of the syndrome On each palm there is a peripheral loop I and the pattern intensity (2 0) is above the mean value of 1 6 for normal females The pattern type I IV e t4 found on both palms of the patient is unusual in English female controls (2/900) Thenar patterns unusual ridge arrangements and higher pattern intensity than in normals are peculiarities observed in most of the patients with trisomy 8

The sole patterns show a high intensity (6 0) are unusual and were not found on either the left or on the right soles in a sample of 250 English controls (100 females+150 males) Zygodactyly occurs on both soles and on the left sole there is a whorl on area III Similar traits are observed on the soles of patients with an extra chromosome number 8 (22 23)

Examination of the blood for coagulation factor VII particularly did not reveal any deficiency of this factor (?8)

DISCUSSION

In the literature 37 cases of trisomy 8 have been reported i e 9 cases of full trisomy 8 (4 6 9 14 17 18 20 27) 19 cases of trisomy 8 mosaicism (1 2 4 6 7 8 11 13 14 15 17

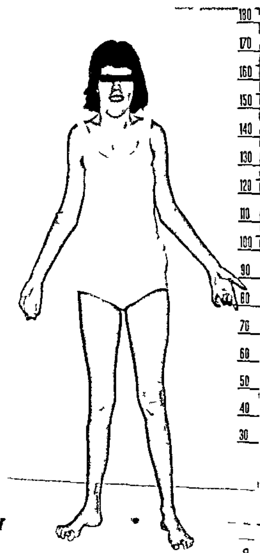






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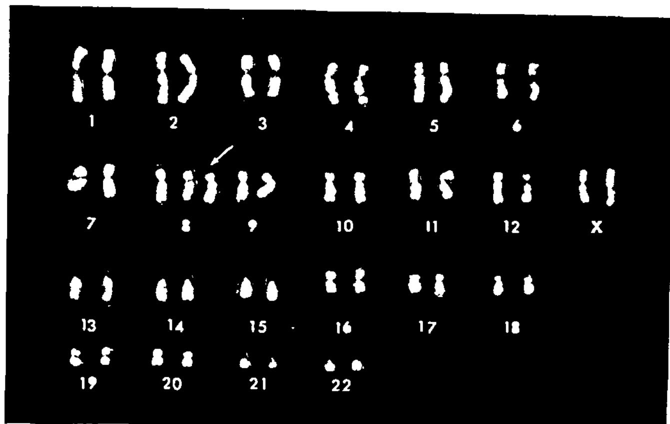


Fig 2 Karyotype 47 XX+del (8)(p 12) (BUDR Acridini Orange staining) Arrow extra chromosome no 8 with partial deletion of the short arm

clustering of signs in any single of the three subgroups There are various reasons for this some reports only summarise the most obvi *ous anomalies under headings such as* dysmorphic appearance and osseous malformations Absence of a specific sign is seldom reported No mention of a parameter tells nothing about whether the sign exists or not it might merely have been overlooked or judged as insignificant Special examinations such as X ray had not been performed in all cases especially if there were no indicative symptom and some of the signs could be a chance finding without correlation to the chromosome anomaly

Nevertheless if the frequencies of each parameter in the three subgroups are added some characteristics of the trisomy 8 syn drome emerge These are in order of de creasing frequency mental retardation seldom of severe degree deformities in the ex tremities especially of the hands and feet de formed and low set ears abnormalities in the vertebral column with spina bifida occulta or butterfly vertebrae restricted articular func *tion in all types of* joints and progressive flex ion contractures with advancing age Skull de formities may be present often with protruding forehead and a broad upturned nose with anteverted nostrils Deep skin furrows of a specific type have been found in most young children with trisomy 8 Bijlsma et al (4) con siders this sign to be age dependant thus fad ing out as the child grows older but the present case does not support this statement as the girl still has deep skin furrows in the soles at the age of 17 Similar findings were reported in a 20 year old male (8) and in a 33 year old female (27) with trisomy 8

Other typical features are the high arched palate sometimes cleft the normal or exces sive body length unusual in other autosomal chromosome trisomies In male subjects cryptorchism is often found

Table 1 *Frequency of clinical signs in various forms of trisomy 8 derived from 38 cases reported in the literature (including present case)*

Parameter	Trisomy 8		Mosaic trisomy 8	Partial trisomy 8	Combined
	B S	Incl B S			
Number described ♂/♀	1	10 (6/4)	19 (15/4)	9 (6/3)	38 (27/11)
Maternal age (?0–4? years)	?9	30 4	29 7	26 7	29 3
Paternal age (1–51 years)	29	31 7	31 4	27 7	30 0
Gestational age (3?–40 weeks)	Full term	33–38 weeks	32–40 weeks	Full term	32–40 weeks
Birth weight (g)	3 000	3 200–4 030	2 050–4 000	2 450–3 500	2 050–4 030
Deformity of skull	×	6/10	6/19	7/9	19/38
Hypertelorism	×	5/10	4/19	2/9	11/38
Strabismus		3/10	7/19	0/9	11/38
Eye deformity		1/10	4/19	2/9	7/38
Ear deformity + low set		6/10	13/19	4/9	?3/38
Low hair limit	×	2/10	1/19	?/9	5/38
Nose deformity broad		3/10	11/19	2/9	16/38
High arched/clefted palate	×	4/10	8/19	3/9	15/38
Micro-retrognathia		4/10	6/19	2/9	1?/38
Long slender trunk/slender pelvis	×	?/10	8/19	4/9	14/38
Spinal deformity		5/10	11/19	5/9	21/38
Rib deformity		4/10	3/19	1/9	8/38
Deformities of extremities	×	9/10	15/19	?/9	31/38
Restricted articular funct	×	5/10	11/19	4/9	20/38
Skin furrows	×	5/10	11/19	1/9	17/38
Cardio-vascular defects		5/10	4/19	1/9	10/38
Urinary tract deformity		3/10	8/19	0/9	11/38
Cryptorchism		1/6	7/15	4/6	12/27
Mental retardation	×	9/10	18/19	9/9	36/38
Agenesia corp callosi			3/19		
Language impairment	×	4/10	7/19		11/38

It may be of interest also that in some cases (13 17) including the present one discrepancies are found in language development e g the impressive language is quite good compared with the expressive language and the child s general level of functioning In the reported cases a preponderance of males is found

In 1974 de Grouchy (14) suggested that a regulatory gene of coagulation factor VII synthesis is localized on chromosome 8 as he found a 50% deficiency of coagulation factor VII in three unrelated patients with trisomy 8 Neither in the present case nor in the 2 cases previously reported by Jacobsen et al (17) could deficiency of factor VII be demonstrated (28)

Observations of mental retardation combined with one or another of the above mentioned clinically easily recognized signs especially the deep skin furrows should lead to a tentative diagnosis of trisomy 8 and to chromosome examinations in order to establish the etiological diagnosis Obvious reasons for this are 1) By means of prenatal chromosome analysis the condition can be prevented in future family members if one of the parents carries a balanced translocation Such cases have been reported by Rosenthal et al (24) and Yanagisawa (30) 2) Prevention and early treatment of contractures which could otherwise be a secondary handicap problem and 3) Further in formation can con tribute to a better phenotypical limitation of the trisomy 8 syndrome

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Submitted July 12 1976
Accepted Oct 30 1976

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LETTERS TO THE EDITOR

Sir

In the article Erythrocyte Glucose 6 Phosphate Dehydrogenase Deficiency (G6PD Type A⁻) and Neonatal Jaundice Bienzle et al (1) express the view that G6PD deficiency is the single most important factor in the pathogenesis of severe neonatal jaundice in the West African population

We would like to bring to your notice that enzymatic deficiency of the liver might be an accessory factor contributing to the severity of the jaundice especially in those cases in which mild haemolysis results in severe hyperbilirubinemia Evidence supporting this view is the decreased salicylamide glucuronide formation (2) and decreased urinary D-glucaric acid excretion during the first week of life (3) The same decrease was observed in G6PD deficient children who developed favism after ingestion of fava beans (4 5)

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The Editor has asked Dr Bienzle and co workers to comment on the letter from Dr Cassimos et al

Sir

We appreciate the point raised by Dr Cassimos and his colleagues Indeed we did not claim in our short paper (1) that neonatal jaundice in the babies we have reported *is* a *direct* consequence of low levels of glucose 6-phosphate dehydrogenase (G6PD) in their erythrocytes we have merely pointed out a strong association between the Gd^{A-} genotype and severe neonatal jaundice in Nigeria The findings in our series (described in more detail elsewhere (2) do actually indicate that in most babies there is little evidence of haemolysis (in agreement with findings from Sardinia (3) Therefore it is entirely possible that the basis for hyperbilirubinaemia lies in the liver The genes responsible for G6PD deficiency in Greece (mainly $Gd^{Mediterranean}$) and in Nigeria (Gd^{A-}) are different (4) We are not entitled to assume that the expression of Gd^{A-} and of $Gd^{Mediterranean}$ in the liver is identical However preliminary data reported in 1975 (5) soon to be published in full indicate that G6PD levels in Gd^{A-} livers are below normal Therefore we feel the suggestion made by Dr Cassimos et al is a possible working hypothesis which we have ourselves entertained it was not raised explicitly in our paper partly for the sake of brevity and partly because the

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Submitted July 12 1976
Accepted Oct 30 1976

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LETTERS TO THE EDITOR

Sir

In the article Erythrocyte Glucose 6 Phosphate Dehydrogenase Deficiency (G6PD Type A⁻) and Neonatal Jaundice Bienzle et al (1) express the view that G6PD deficiency is the single most important factor in the pathogenesis of severe neonatal jaundice in the West African population

We would like to bring to your notice that enzymatic deficiency of the liver might be an accessory factor contributing to the severity of the jaundice especially in those cases in which mild haemolysis results in severe hyper bilirubinemia Evidence supporting this view is the decreased salicylamide glucuronide formation (2) and decreased urinary D glucaric acid excretion during the first week of life (3) The same decrease was observed in G6PD deficient children who developed favism after ingestion of fava beans (4 5)

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Chr Cassimos
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J Tsiuris
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The Editor has asked Dr Bienzle and co workers to comment on the letter from Dr Cassimos et al

Sir

We appreciate the point raised by Dr Cassimos and his colleagues Indeed we did not claim in our short paper (1) that neonatal jaundice in the babies we have reported is a direct consequence of low levels of glucose 6 phosphate dehydrogenase (G6PD) in their erythrocytes we have merely pointed out a strong association between the Gd^{A-} genotype and severe neonatal jaundice in Nigeria The findings in our series (described in more detail elsewhere (2) do actually indicate that in most babies there is little evidence of haemolysis (in agreement with findings from Sardinia (3) Therefore it is entirely possible that the basis for hyperbilirubinaemia lies in the liver The genes responsible for G6PD deficiency in Greece (mainly $Gd^{Mediterranean}$) and in Nigeria (Gd^{A-}) are different (4) We are not entitled to assume that the expression of Gd^{A-} and of $Gd^{Mediterranean}$ in the liver is identical However preliminary data reported in 1975 (5) soon to be published in full indicate that G6PD levels in Gd^{A-} livers are below normal Therefore we feel the suggestion made by Dr Cassimos et al is a possible working hypothesis which we have ourselves entertained it was not raised explicitly in our paper partly for the sake of brevity and partly because the

mechanism whereby a lower than normal G6PD level in the liver can lead to jaundice in the newborn is not yet clear

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REFERENCES

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NEW BOOKS RECEIVED

B Bobath *Abnormale Haltungsreflexe bei Gehirn schaden* 3rd ed 94 pp illus Georg Thieme Verlag Stuttgart 1976 DM 9 80

W Blunck *Pädiatrische Endokrinologie Hormone Wachstrum Pubertat* 360 pp illus Urban & Schwarzenberg Munchen Wien Baltimore 1977 No price given ISBN 3 541-07471 X

P Catzel *A short textbook of paediatrics* 446 pp illus Hodder & Stoughton London 1976 £3 45 (paperback) ISBN 0-340-05109-4

M Coleman (ed) *The autistic syndromes* 334 pp illus Elsevier North Holland Publishing Company Amster dam 1976 US$3? –

M Cornblath & R Schwartz *Disorders of carbohydrate metabolism in infancy* 2nd ed 501 pp illus *In* A J Schaffer & M Markowitz (eds) Major problems in clinical pediatrics vol III W B Saunders Company Philadelphia London Toronto 1976 No price given ISBN 0-7216-27?1-8

G R Fraser *The causes of profound deafness in child hood A study of 3 535 individuals with severe hearing loss present at birth or of childhood onset* 429 pp illus Bailliere Tindall London 1977 £17 00 ISBN 0-70 0-0640-8

J A Kuzemko (ed) *Asthma in children* 1? pp illus Pitman Medical Publishing Co Ltd London 1976 £3 50 ISBN 0 272 79400 7

Zvi Laron (ed) *The adipose child* vol 1 ??7 pp illus *In* Z Laron & Z Dickerman (eds) Pediatric and Adolescent Endocrinology S Karger Basel Munchen Paris London New York Sydney 1976 sFr/DM 89 – ISBN 3 8055 343

J-C Laroche *Developmental pathology of the neonate* 545 pp illus Elsevier Excerpta North Holland Amsterdam New York 1976 US$73 50 ISBN 90 ?19 ?107 3

L O Lubchenco *The high risk infant* vol XIV 294 pp illus *In* A J Schaffer & M Markowitz (eds) Major problems in clinical pediatrics W B Saunders Company Philadelphia London Toronto 1976 £15 00 ISBN 0-7216-5800-8

R I Mackay *Mental handicap in child health practice* 3?? pp illus The Butterworth Group London 1976 £9 50 ISBN 0-407-00113 J

J L Melnick (ed) *Progress in medical virology* Vol ?? 230 pp illus S Karger Basel Munchen Paris Lon don New York Sydney 1976 sFr/DM 119 — ISBN 3-8055 2315 7

G R Osborn & N Roydhouse *The tonsillitis habit* 100 pp illus W P Roydhouse Publishers Auckland 1976 $12 00

S H Pierog & A Ferrara *Medical care of the sick new born* 2nd ed 368 pp illus The C V Mosby Company Saint Louis 1976 US $14 50 ISBN 0-8016-3936-0

D M Ross & S A Ross *Hyperactivity Research the ory and action* 385 pp John Wiley & Sons Ltd Chi chester 1976 £13 75 ISBN 0-471 73678 3

F H Stone *Psychiatry and the paediatrician* 175 pp *In* J Apley (ed) Postgraduate paediatrics series Butterworths London Boston 1976 £6 00 ISBN 0-407-00074 7

E B Weiss & M S Segal (eds) *Bronchial asthma* 1168 pp illus Little Brown and Company Boston 1976 US$50 00 ISBN 0-316-9?886-0

A W Wilkinson (ed) *Early nutrition and later develop ment* ?36 pp illus Pitman Medical Publishing Co Ltd London 1976 £8 00 ISBN 0-??? 79411 ?

D S Young & J M Hicks *The neonate Clinical bio chemistry physiology pathology* 340 pp illus John Wiley & Sons Ltd Chichester 1976 £14 15 ISBN 0-471 9798? 1

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The second part of the book gives detailed information of the clinical picture and the treatment of separate types of tumours In most cases it presents didactic and informative descriptions of the nature of the tumours and brings detailed information of schemes of therapy The great experiences of the authors and the large clinical materials presented in the book will guarantee that the aspects given are conclusive and of good advice to the reader

The subject index is comprehensive and clearly edited and the illustrations and therapy schemes are very instructive Indeed this book is a stimulating practical important and generous up-to-date guide to all doctors (pediatricians surgeons radiotherapists pathologists) working in the field of pediatric oncology

Anders Kreuger

K Z Štembera K Polacek & V Šabata (eds) *Perinatal medicine* 4th European Congress of Perinatal Medicine Prague August 1974 556 pp illus Georg Thieme Publishers Stuttgart Avicenum Czechoslovak Medical Press Prague 1975 DM 98 – ISBN 3 13 5?9101-4

The editors declare that The final aim of the Proceedings is to present to the reader a survey of contemporary knowledge in the three main topics that were dealt with at the Congress This volume therefore contains the full text of the three panels dealing with Antenatal diagnosis of the at risk fetus Premature delivery and the preterm infant and The short and long term prognosis of perinatal complications In doing this the editors succeed very well Of the numerous free communications only those relating to these main themes have been included On 539 pages the reader will find a condensation product of 1974 vintage in different European centers as regards research in these major areas of perinatal medicine

A great number of illustrations tables and references as well as subject and author indexes increase the value of this volume which is clearly of great interest to all neonatologists

Olle Celander

F C Battaglia G Meschia & E J Quilligan (eds) *Perinatal medicine Review and comments* vol 1 146 pp illus The C V Mosby Company Saint Louis 1976 US$19 45

This book is a review and selection of the literature in neonatal perinatal medicine for the year 1974 It is divided in 16 chapters dealing with various aspects of perinatal medicine Pertinent papers within each field are shortly presented and commented upon by the editors with the aim to provide guidelines for an approach to this widely scattered literature for residents and postdoctoral fellows

Editor

D Fochtman & J G Raffensperger *Principles of nursing care of the pediatric surgery patient* 2nd ed Little Brown and Co Boston 1976 US$1? 50 ISBN 0-316- 8681-8

The book consists of twelve chapters dealing with the nursing care in various paediatric surgical disorders In the Foreword and Preface the role played by the nurse in diagnostics and pre and postoperative care is defined It is also stressed that the nurse has a great responsibility of her own e g to support parents of defect or handicapped children

Every chapter gives a short description of a paediatric surgical disorder In addition to the general surgical disorders the book also treats specialized paediatric surgery i e thoracic plastic and orthopaedic surgery as well as neurosurgery otological and ophthalmological surgery Each chapter is independent of the others which is an advantage for teaching purposes The pre and postoperative care in each of the conditions is presented point by point The presentation is clear and comprehensive

The pictures are of good quality and are up to date and the illustrations are clear

The need for psychological care of the whole family is repeatedly stressed especially in the chapters on burns congenital heart disease and facial malformations

Much in the book can be applied to conditions in the Scandinavian countries However from our point of view the value of the book would have been greater if it had also *included* a chapter on the cooperation between different members of the nursing staff and on how to delegate the various tasks in the nursing care Still the book should be of value in the training of nurses and on paediatric surgical wards

Kerstin Syrehn

W Schroter G Prindull & U Kaehler *Blutkrankheiten im Kindesalter* Urban & Schwarzenberg Munchen 1976 144 pp illus

This book written in German is intended as an up to-date survey of the diagnosis and treatment of haematological diseases in childhood The text is addressed to general practitioners as well as to paediatric specialists After an introductary survey of normal blood parameters including blood physiology separate chapters deal with disorders of the erythrocytes haemostasis and the leukocytes including leukemias lymphomas and reticuloendothelial diseases

With regard to the comparatively few pages of the book much information is given and most disorders and many eponymas are mentioned Especially the part concerning the erythropoietic system is clearly and thoroughly written somewhat in contrast to the other parts of the book The brief compendium style of the book necessitates more references than are now given

It is probable that doctors who are not commonly faced with paediatric haematological problems would appreciate chapters that are missing e g a survey of the rapidly expanding field of immunology of methodological problems in laboratory diagnosis and of attempts to systematize the investigations needed in different clinical situations

However the book serves as a good introduction to paediatric haematology and as a complement to textbooks in general paediatrics

Anders Kreuger

BOOK REVIEWS

Lytt I Gardner (ed) *Endocrine and genetic diseases of childhood and adolescence* W B Saunders Company Philadelphia London Toronto 1975 1403 pp 2nd ed

Six years have passed since the first edition of this text book appeared During this relatively short period there has been a rapid expansion of scientific knowledge especially in the field of genetic diseases This is *inter alia* reflected in this book by an increase in the number of pages from 1072 to 1403 Most of the chapters have been thoroughly revised and amplified The chapter on prenatal genetic diagnosis is entirely new and the contributions on psychologic aspects of genetic and endocrine diseases and on cytogenetics are greatly expanded A new classification of patients with ambiguous genitalia has been introduced It is certainly a matter of opinion how much information that should be included in a text book of this type The editor states that the purpose *of the book is to provide a definitive medical textbook* oriented to the primary care physician whether he be pediatrician internist gynecologist or family physician This has no doubt been achieved the book is in many ways excellent and can be highly recommended to the categories of readers mentioned but one may for instance ask if a detailed description how to prepare peripheral blood leukocyte cultures really ought to be included

C C Bergstrand

Moshen Ziai (ed) *Pediatrics* 1021 pp illus Little Brown & Co Boston 1975 No price given ISBN 0-316 98751-4(P)

This book is a compendium of pediatrics addressed to medical students and physicians It is a voluminous compendium of 1071 pages In the introduction the editor underlines the great diversity in growth conditions for children in developing and industrial countries He states that it is time for medical education to pay proper attention to priorities if we will get healthier children and happier nations

After this declaration one starts reading with great interest However one finds a text book of conventional type The book has become voluminous in spite of the fact that not much room has been given to deep-reaching discussions Clinical rarities are placed side by side with more common diseases which makes it difficult for a medical student to see what is really essential

In spite of the global aspects in the introduction much of the book reflects conditions in the USA Insufficient space is devoted to preventive aspects and social pediatrics The book also lacks a thorough discussion of psychological factors around the sick child and its family Nutrition is poorly dealt with Not even in the chapter on obstipation is the importance of the diet mentioned Breast feeding is not given much attention Such important fields as the battered child syndrome and sudden unexpected infant death are just touched upon

The largest part of the book is devoted to the various diseases The chapter on infectious diseases has the character of a textbook Tuberculosis has got 15 pages In contrast such an important disease as the hyaline membrane syndrome is dealt with in less than one page and all that is said about CPAP treatment is that more recently constantly maintained pulmonary inflation has saved many lives which does not give much basis for practical clinical work Allergic diseases have also been given insufficient space In the treatment of bronchial asthma one misses some modern therapeutic methods e g prophylaxis with dichromoglycate

The book ends with some appendices which treat the differential diagnosis in common clinical conditions such as unconsciousness vomiting fever seizures This chapter is perhaps the most valuable in the book In the last appendix there is a table of normal values which however is not adapted to the SI system This is true of the whole book which makes it less useful in most West European countries

Lennart Righard

H J G Bloom J Lemerle M K Neidhardt & P A Voute (eds) *Cancer in children Clinical management* 317 pp illus Springer Verlag Berlin Heidelberg New York 1975 ISBN 3 540-07261 6 US $14 20

The rapidly expanding field of pediatric oncology demands repeated survey in order to bring accumulated experience and expertise to the doctors treating childhood cancer On behalf of the Patient Care Committee of the International Union Against Cancer (UICC) 45 authors from 8 countries have collaborated and contributed to this book which due to the excellent work of the editors brings actual and thorough information on the clinical management of cancer in children

The first part of the book gives general aspects of malignant diseases as aetiology pathology and the principles and the background of different ways of treatment However a survey of the interesting recent immunological aspects on the nature of malignant diseases is lacking The book clearly emphasizes the importance of a good team work between the specialists engaged in the treatment of childhood cancer

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ABSTRACT Köhler L and Holst K (Department of Paediatrics University Hospital Lund Sweden) Dental health of four year-old children Acta Pædiatr Scand 00

An unselected population of 1 567 four year old hildren

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1 Brecher G & Stohlman F, Jr Humoral factors in erythropoietin. *In* L M Tocantins & R Penn (eds.) *Progress in hematology* Grune & Stratton, New York 1959 p 110
2. Smith C A *The physiology of the newborn infant* Thomas, Springfield, Ill. 1967 3rd ed vol. 2 p 120
3 Vedra, B & Ulrych, J. Anaerobiosis in normal and asphyxiated premature newborns. 2. Four approaches *Acta Paediatr Scand* 49 129 1960

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G H Valentine *The chromosome disorders An introduction for clinicians* 3rd ed William Heinemann Medical Books Ltd London 1976 184 pp illus £5 00

This book is written by the professor of paediatrics at the University of Western Ontario Canada and dedicated to Dr Barr of the same university who initiated the science of clinical cytogenetics by his discovery of the X chromatin (Barr) body in 1949 This book is a well written brief review divided into two parts Part I The grammar of cytogenetics and Part II The chromosome diseases

The strength of the book lies firstly in the fact that the author is writing from extensive personal practical clinical and cytogenetical experience and secondly in the excellent illustrations The author has written in a narrative even conversational and humorous style The presentation progresses step by step from the biological facts to the more complex clinical picture

This is a book which one would especially recommend paediatricians in training

Karl Henrik Gustavson

Gordon Brocklehurst (ed) *Spina bifida for the clinician* Clinics in Developmental Medicine No 57 Spastics International Medical Publications William Heinemann Medical Books Ltd London 1976 195 pp illus £5 50

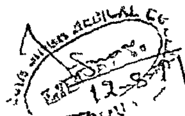
D Forrest W J W Sharrard and G Stark are the wellknown co authors of this new monograph dealing with the complex care of the child with spina bifida The vast experience of these authorities from Great Britain guarantees the high quality of the material presented and the solid foundation for the opinions expressed The outstanding chapter on the general paediatric management by Stark contains much good advice on how to handle the patient and the parents in the common difficult situations The special section on the management of bladder and bowel by Forrest is also a valuable guide for the physician directly involved in the care of spina bifida children The advice to treat mild cases of recurrent symptomatic urinary infections with a small dose of a suitable drug at bedtime is however based on a study of urinary tract infections in adults with non neurogenic bladder disturbances and is hardly valid for the child with myelomeningocele

The tremendous experience by Sharrard is reviewed in his chapter on the general and specific orthopaedic management What one is perhaps missing in this as well as in almost all other monographs on spina bifida is a section containing physiotherapy and physical training in general which is of greater importance than many physicians realize The original work of the editor on the pathology of spina bifida and the associated hydrocephalus as well as the relationship of pathological lesions to clinical manifestations is extensively presented perhaps a little out of proportion for a volume of this character The book ends with a survey of the results of treatment and of the selection of the type of treatment and it is pointed out that the *difficult decision to operate or not should be made individually* for each patient and should not be just a result of gross categorization

Bo Hellstrom

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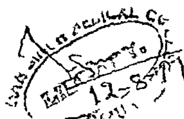
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constit. et color (titandioxid) q s.
Mixtur 25 mg/ml 1 ml innehåller erythromycin stear respond erythromycin 25 mg saccharum 0 7 g constit. color (para-orange) et aroma q s aq purif ad 1 ml

Egenskaper Reciomycin har ett spektrum omfattande framst grampositiva bakterier men aven vissa gramnegativa patogena organismer Som exempel på kansliga organismer kan namnas streptokocker (inkl. β hemolytiska grupp A) stafylokocker (inkl penicillinasbildande) pneumokocker Corynebacterium, Neisseria och Mycoplasma pneumoniae.

Reciomycin ar ej verksamt mot E coli, Proteus eller Pseudomonas Preparatet har mycket ringa tendens att forandra den normala tarmfloran.

Indikationer Infektioner orsakade av erytromycinkansliga mikroorganismer Till penicillinoverkansliga patienter kan Reciomycin anvandas som ersattningspreparat till penicillin G eller V

Reciomycin tillhor forstahandspreparaten vid behandling av mykoplasmainfektioner

Kontraindikationer Kand erytromycinoverkänslighet

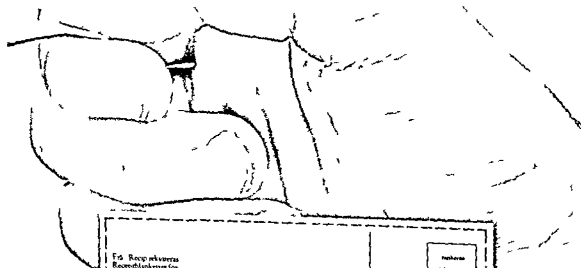
Biverkningar Illamående och latta gastrointestinala besvar kan forekomma. Allergiska reaktioner ar sallsynta uppskattningsvis i 0,3% av behandlade fall

Dosering
Vuxna Normaldos 0,5 g morgon och kvall (var 12 e timme) alternativt 0 25 g 4 gånger per dygn Då hogre serum- och vavnadskoncentrationer onskas ges 0,5 g 3–4 gånger per dygn.
Barn Normaldos 40 mg (=1 6 ml) per kg kroppsvikt och dygn fordelat på 2–4 doseringstillfallen.

Doseringsschema for mixtur.
1/2–5 år 1–2 dosskedar (5–10 ml) 3 gånger per dygn
5–8 år 2–3 dosskedar (10–15 ml) 3 gånger per dygn
For att uppnå optimal absorption bor Reciomycin helst tas 1 timme fore eller minst 2 timmar efter måltid

Forpackningar. Tabletter (vita, kapselformade filmdragerade, ca 9×17,5 mm) 40 st och 100 st Mixtur (fardigberedd) 150 ml

AB Recip 10425 Stockholm tel 08/520235



Acta Pædiatr Scand 66 129–132 1977

EDITORIAL

A SWEDISH CODE OF ETHICS FOR MARKETING OF INFANT FOODS

A decline of breast feeding has been observed as a world wide phenomenon in recent decades Most unfortunately this tendency has spread to large population groups which like the urban slum dwellers of Third World countries lack even the basic requirements (finance hygiene education etc) for successful artificial feeding at an early age

This development has caused growing concern among health professionals as well as in various organizations working in the health and nutrition field At the World Food Conference in Rome in 1974 (8) Resolution V dealt with this problem The following recommendation is a quotation of item 5 6

> That governments consider the key role of women and take steps to improve their nutrition their educational levels and their working conditions and to encourage them and enable them to breast feed their children

The underlying reasons for the decline in breast feeding in many parts of the world are diverse and complex Some of the major ones are social change (the extended family giving way to the two-generation one) desire for independence increased engagement of women in professional work outside the home inexperience and inertia of health personnel attraction of breast milk substitutes promoted through unduly persuasive advertising An additional contributory factor especially in the Third World countries is the example set by members of higher social classes who mimic Western behavioral patterns

In this context only the adverse effects of unhampered promotion of industrially produced infant formulas will be dealt with This problem complex has attracted increasing attention during the 1970 s

A consecutive series of meetings between representatives of the infant food industry and paediatricians has been organized under the auspices of the UN Protein Calorie Advisory Group (PAG) The most recent outcome of these deliberations is found in PAG Bulletin 1975 V 1 (6) Recently the infant food industry itself has taken the initiative of organizing an International Council of Infant Food Industries (3) which has presented its own code of ethics

In addition to the above other bodies have commented in more general terms upon the ethics of promotion of infant foods Thus at the 13th International Congress of Pediatrics in Vienna 1971 the International Pediatric Association (IPA) organized a fullday work shop on New urban families (4) which among its recommendations included the following

> Realizing the paramount importance of good nutrition during early life especially during the first six months and that under the conditions prevailing among underprivileged groups in developing countries it is extremely difficult to substitute any suitable food for breast milk the formula producing food companies should observe great caution in applying methods of promoting their products They (persons contacting mothers) should never use their influence to promote a particular product in such a way that it could be detrimental to good breast feeding practices

Likewise WHO at the 27th World Health Assembly in 1974 in a Statement on Infant Nutrition and Breast feeding (2)

Urges member countries to review sales promotion activities on baby foods and to introduce appropriate remedial measures including advertisement codes and legislation where necessary

And more recently at an IPA seminar at Montreux (August 1975) for paediatricians from N Africa and Middle East Recommendations for Action Programs to encourage Breast feeding were adopted (7), these also including a section Curtailing Promotion of Artificial Feeding which deserves to be quoted in extenso

Sales promotion activities of organizations marketing baby milks and feeding bottles that run counter to the general intent expressed in this document must be curtailed by every means available to the profession including where necessary and feasible legislation to control unethical practice

Dissemination of propaganda about artificial feeding and distribution of samples of artificial baby foods in maternity units should be banned immediately

It is quite obvious that we are still far from achieving a proper balance between what can be regarded as acceptable business activities on the one hand and social and nutritional considerations on the other No wonder then that the multinational corporations in the infant food business have been subject to growing criticism (1)

In this period when the marketing ethics of the infant food industry are under sharp scrutiny it would seem that any relevant experience of attempts to monitor the activities in this field is worth imparting to a wider circle This Journal has therefore deemed it of interest to present the following document Medical standards for marketing of infant foods , which was worked out in Sweden as early as in 1964 as a guide to the infant food industries

Instrumental in preparing this code was a group of paediatricians the majority of them professors of paediatrics at Swedish universities As consultants to the two leading Swedish infant food industries they have been in the position to monitor the implementation of the code It can be clearly stated that the code has not remained just a pious declaration It has indeed been pivotal in avoiding aberrations detrimental to the consumer

On the whole the Swedish industries concerned should be given great credit for having shown a clear ambition to follow the standards laid down in the code thus proving their understanding for the concern of the paediatricians and the need of a marketing approach which does not undermine the mothers interests in breast feeding their children

In 1975 some slight revisions of the code were introduced but basically and in all its salient points the revised edition which is presented below closely follows the original one

It may be of some interest to note that the rules laid down in the Swedish code and the marketing procedures thus adhered to are much more detailed and strict than those in any of the codes or recommendations discussed above The consequences of unethical marketing in Third World countries are potentially more harmful than in a Scandinavian country Thus it would seem that the standards of the Swedish code adapted to the situation of Third World countries would form a sound basis for the discussion of their needs

Third World countries also need to have access to infant formulas—industrially produced or not—for those infants who suffer from a definite undernutrition at the breast As long as the undernutrition is of a mild degree it is however certainly better not to interfere For children from the dominating groups of poor families it is better to be exposed to a period of slight undernutrition than to run the risk of infections and diarrhoeal diseases following administration of formulas Further in industrial countries semisolid and solid baby food (so called Beikost) is normally recommended to be introduced at 3–4 months but in Third World countries for the reasons just mentioned it should rather be started at 6 months if breast feeding is reasonably satisfactory

But then we have also in Third World coun

tries mothers who at an early stage and in spite of a strong motivation to breast feed dry up In these cases and if a wet nurse cannot be recruited the infants must be given some kind of formula sometimes before the age of 6 months These babies should be carefully supervised by the local health centre The formula to be used in these cases should preferably be of a simple milk based nature or possibly some kind of stretch the milk type (mainly vegetable) product (5) In either case the procurement of such an infant food would be beyond the means of a poor family Thus the survival of the child will be largely dependent on institutional feeding programme with free distribution through proper health service channels

It must be acknowledged that such programmes with free distribution of infant formula products commercial or non commercial (through government national and international voluntary organizations etc) must be carried out in a very careful way if they are not to come rightly under the same criticism as the baby food industry *It is true that the financial* problem may be thus overcome but they may lead to a flooding with formula products a displacement of breast feeding and all the risks of contamination that prevail under unhygienic environmental conditions This aspect of institutional infant feeding programmes has been given too little attention in the past

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5 Protein-rich mixtures for use as weaning foods PAG Guideline no 8 1972
6 Recommendations on Policies and Practices in Infant and Young Child Feeding and Proposals for Action to Implement Them *PAG Bulletin 5* 1 1975
7 Recommendations for Action programs to encourage breast feeding *IPA Bulletin* 1975 No 4 19 Also published in *Acta Paediatr Scand 65* 275 1976
8 World Food Conferen e Resolution V Policies and programmes to improve nutrition Rome 1974

MEDICAL STANDARDS FOR MARKETING OF INFANT FOODS IN SWEDEN[1,2]

Version Released by a Group of Swedish Paediatricians 1975[3]

1 Advertisements for and information concerning infant foods should always be in a form which is concordant with the views on infant feeding held by the medical consultants of the firms in question

2 Advertisements for breast milk substitutes (starting infant formulas) should in no form be aimed directly towards the public or towards individual families Special discount offers to the consumer should not be made with regard to products of this type

3 Advertisements sent directly to the individual family for other infant foods such as fruit juices baby drinks strained and junior foods follow up formulas and similar foods should not be distributed so that they reach the consumer before the child is about 3 months old

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4 Printed matter with qualified contents such as Mitt barn (My child) and Babys egen bok (Baby s own book) should only be distributed through doctors and nurses It should be permissible, however for the firms concerned to send printed matter of this kind to individual persons on special request Simple printed matter such as lists of infant food assortments should however, be allowed to be distributed directly to the consumer but never so that they reach the consumer before the child is about 3 months old

5 In printed menus intended for the public for infants under 4 months of age only breast milk should be given as the main component of the meals A comment may indicate, however that the Child Health Centres will give information on appropriate substitutes in cases where breast milk is deficient or lacking

6 Demonstrations of infant food products or instructions on infant feeding for example in association with Mothers evenings may be arranged by the infant food industry provided that they take place in collaboration with a doctor or registered nurse

7 All new booklets or other printed matter directed towards the public should be scrutinized by the respective medical consultants before publication The same should apply to advertisements in the daily and weekly press in medical journals and journals for nurses and to all informational material that is to be distributed to doctors nurses, hospitals children s homes and Child Health Centres etc

8 Free distribution of infant food and similar promotion measures for purposes of advertisement should not occur

9 By advertisement in the above is meant all forms of advertising (in professional journals daily newspapers, weekly magazines books such as Vi Foraldrars Baby bok (The parents baby book) etc , posters in stores booklets lists of food assortments books on infant foods and the like

Reprints of the article may be obtained from the Swedish Pediatric Association c/o Dr Orvar Finnström Department of Paediatrics University Hospital S 58185 Linkoping Sweden

Acta Pædiatr Scand 66 133–135 1977

BILIRUBIN DISPLACING EFFECT OF STABILIZERS ADDED TO INJECTABLE PREPARATIONS OF HUMAN SERUM ALBUMIN

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ABSTRACT Brodersen R and Hansen P (Institute of Medical Biochemistry Aarhus University Aarhus and Statens Seruminstitut Copenhagen Denmark) Bilirubin displacing effect of stabilizers added to injectable preparations of human serum albumin Acta Paediatr Scand 66 133 1977.—Stabilizers added to preparations of human serum albumin before heat treatment were tested for bilirubin displacing effect using the peroxidase method It was found that N-acetyltryptophan and sodium caprylate displace bilirubin from its complex with human serum albumin *in vitro* The quantitative findings were used for a rough estimate of the effect of these substances on the free bilirubin concentration in blood plasma expected when stabilized albumin preparations are given intravenously for prevention of kernicterus The calculated effect is a delay of the decrease of free bilirubin concentration or even a temporary increase Sodium mandelate displaces less strongly

KEY WORDS Albumin bilirubin displacement fatty acids kernicterus stabilizers

Human serum albumin is often used before or during exchange transfusion in prevention of kernicterus The amount of bilirubin removed from the body is substantially increased if an intravenous dose of albumin is given 1–2 hours before exchange (7) In the manufacture of injectable albumin preparations stabilizers are added to protect the protein during heat treatment 10 hours at 60° used to prevent transmission of hepatitis virus Accepted stabilizers are the sodium salts of N acetyltryptophan or caprylic acid or a mixture of both in a total concentration of 0 04 mol/l (6 8) Sodium mandelate has been used elsewhere to a limited extent The possible effect of giving these compounds together with the albumin has received little attention It has previously been shown (2) that N acetyltryptophan may displace bilirubin *in vitro* from its complex with serum albumin The present work extends these investigations to the other two stabilizers The results are used for a tentative calculation of the effect of these substances on the concentration of free bilirubin in the blood plasma

MATERIALS AND METHODS

Human serum albumin was from KABI Sweden This preparation does not contain added stabilizers and has not been heated The peroxidase method was used as previously described (?) to measure bilirubin displacing effect

RESULTS

The results are seen in Fig 1 The graph shows the velocity of oxidation of bilirubin 15 μmol/l with hydrogen peroxide and peroxidase in the presence of human serum albumin 30 μmol/l This velocity increases with the concentration of added stabilizer indicating

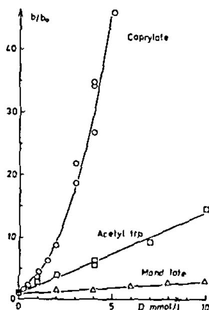


Fig 1 Displacing effect of stabilizers on bilirubin bound to human serum albumin measured by the peroxidase method (2) Ordinate relative rate of oxidation of bilirubin This is proportional to the free i e non protein bound bilirubin concentration Abscissa concentration of the displacer in the reaction mixture Bilirubin concentration was 15 μmol/l human serum albumin 30 μmol/l phosphate buffer 60 mmol/l pH 7 4 37 O Sodium caprylate □ N acetyltryptophan sodium salt Δ sodium mandelate

that albumin bound bilirubin is made available to oxidation by the enzyme In case of simple competitive displacement of bilirubin by the added substance a straight line is obtained (2) as seen for N acetyltryptophan and mandelate The curved line for caprylate indicates that a more complicated mechanism is operative with high concentrations of this substance possibly involving a conformational transition of the albumin molecule Control experiments carried out in the absence of albumin, showed that none of the stabilizers reacted directly with bilirubin nor with the reagents used in the test The free bilirubin concentration is therefore proportional to the oxidation velocity and can in relative terms be read from the ordinates of the curves

It is seen that all three substances displace bilirubin to some extent from its binding to human serum albumin The effect increases in the order mandelate acetyltryptophan caprylate

DISCUSSION

The possible significance of these findings when stabilized albumin preparations are used in the treatment of icteric neonates for prevention of kernicterus may be roughly estimated as follows

An albumin dose of 1 g/kg given as a 20% solution with 40 mmol/l N acetyltryptophan to an infant with a plasma volume of 40 ml/kg would give an added albumin concentration about 340 μmol/l and an initial concentration of 4 4 mmol/l of the stabilizer when mixed completely with the blood plasma Most of the stabilizer about 4 mmol/l would be present as non protein bound N acetyltryptophan and would as seen from the figure cause a 6 fold increase of free bilirubin concentration This would be counteracted by binding of bilirubin to the albumin given The magnitude of the latter effect would vary with the concentrations of bilirubin and albumin before treatment Since the free bilirubin concentration is inversely proportional to the molar surplus of albumin over bilirubin and if the concentration of albumin is between 27 and 30 g per liter (410 to 450 μmol/l) and bilirubin is from 18 to 22 mg per 100 ml (310 to 380 μmol/l) the molar surplus of albumin ranges from 30 to 140 μmol/l before treatment and from 370 to 480 μmol/l after the albumin dose The albumin surplus accordingly increases by a factor roughly between 3 and 12 and the free bilirubin concentration decreases by a factor between 3 and 12 as a result of giving albumin alone The total outcome after giving N acetyltryptophan and albumin together is therefore likely to range from an increase of free bilirubin concentration by a factor 2 to a decrease to half of the level before treatment During a period of time after the injection the stabilizer is eliminated from the blood stream and a decrease of free bilirubin concentration may be expected

Sodium caprylate is a salt of a medium chain fatty acid Its strong effect in the experimental procedure is moderated *in vivo* by binding to the albumin molecule where it is mostly ac

comodated in sites which do not bind bilirubin As estimated from the binding isotherm published by Ashbrook et al (1) about 70% is protein bound at the actual concentration level This means that the displacement caused by caprylate in the blood plasma would be of the same order of magnitude as that of N acetyltryptophan Special caution is however indicated with this substance if increased amounts of fatty acids are already present before treatment As shown by Ashbrook et al binding of caprylate is decreased by the presence of long chain fatty acids The concentration of these is often somewhat elevated in newborns (3) and may be very high in case of generalized infection (4) or after use of intravenous lipid preparations for parenteral nourishment (5 10) The steep curvature of the line in Fig 1 suggests a potentiated effect of high concentrations of fatty acids in agreement with previous observations (9) Also slow elimination in a premature child already loaded with fatty acids would further delay the onset of the bilirubin binding process

Mandelate displaces considerably less The efficiency of this substance as a stabilizer and the possible pharmacological effects of the large doses in question have not been investigated in sufficient detail

In conclusion determination of the bilirubin displacing effect of stabilizers *in vitro* seems to indicate that N acetyltryptophan and caprylate if present in permitted amounts in injectable preparations of human serum albumin may cause a delay or even a temporary reversal of the bilirubin binding effect of the albumin given Removal of the stabilizers before dispensing would result in preparations without potential bilirubin-displacing capability and presumably with a more prompt bilirubin binding effect The capability of the preparations to bind bilirubin could be tested by the peroxidase method Such testing would further ensure that the full effect of the albumin has been restored after heat treatment

ACKNOWLEDGEMENT

The skilful technical work of Signe Andersen is acknowledged

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Submitted June 14 1976
Accepted Sept 7 1976

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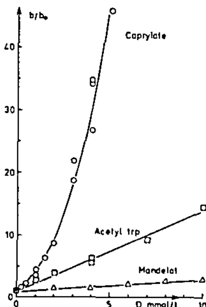


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Submitted June 14 1976
Accepted Sept 7 1976

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Acta Pædiatr Scand 66 137–143, 1977

LONG TERM EFFECT ON MOTHER–INFANT BEHAVIOUR OF EXTRA CONTACT DURING THE FIRST HOUR POST PARTUM

I *First Observations at 36 hours*

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ABSTRACT de Chateau P and Wiberg B (Department of Paediatrics and Department of Child Psychiatry University of Umeå Umeå Sweden) Long term effect on mother–infant behaviour of extra contact during the first hour post partum I First observations at 36 hours Acta Paediatr Scand 66 137 1977.—The immediate post partum period may be particularly important for the developing relationship between mother and infant little is as yet known however of the long term effects of hospital practice during this period This study examines the effect of extra contact during the first hour following delivery An extra skin to skin contact and suckling contact was given to 22 primiparous mothers and their infants One control group of 20 primiparous mothers and infants and a second one of 20 multiparous mothers and infants was given routine care immediately after birth All mothers and infants were healthy with normal pregnancies and deliveries At 36 hours a first observation was made of maternal and infant behaviour during breast feeding in all three groups At this stage primiparae with extra contact showed behaviour much more like the behaviour of multiparae with routine care Infants of primiparae with routine care cried most frequently The behaviour of mothers of boys differed more from group to group than did that of mothers of girls

KEY WORDS Mother–infant behaviour extra contact neonatal period observations

An extended contact between mother and infant during the first post partum days may influence development of mother–infant interaction and attachment (4 6) Klaus et al (4) in their study gave to 14 mothers one hour of close physical contact with their nude fullterm infants within the first three hours after delivery and another 15 extra hours of contact in the first three days post partum (extended contact) a control group of mothers was given routine contact with their newborn babies In follow up studies one month (4) one year (5) and two years (16) after delivery differences between the extended contact and the control group were found with regard to maternal attachment behaviour and linguistic behaviour Maternal and infant behaviour is controlled by many other factors than early physical contact only Prenatal influences like the mother s relationship with her own mother (12) and the infant s father her cultural background planning and preparations for pregnancy delivery and so on may be of great importance and must therefore be checked when studies of the impact of changes in the immediate post partum period on maternal and infant behaviour are presented

In this study we have examined what effect extra contact limited to 15 min immediately following delivery might have on mother and infant behaviour Three groups of mother–infant pairs were studied 22 primiparae and their infants with during the first hour after delivery extra naked skin to skin and suck

METHODS

Routine care immediately following delivery (P and M groups)

After delivery the baby lies on the delivery table between the legs of the mother Mouth and upper airways are rinsed and the stomach emptied Face trunk and legs are wiped dry with a towel The infant is then shown to the mother for a brief glance but usually she does not touch him A numbered bracelet is put around the wrist of both mother and infant After cord clamping 2–6 min post partum the baby is taken to another part of the delivery room for weighing bathing physical examination Crede prophylaxis and dressing This takes approximately 30 min In the meantime the mother is helped to deliver the placenta washed and cleaned The baby—with clothes on—is put in a crib and covered with a blanket The crib is placed beside the mother s bed so that she can watch her baby and touch the face In some instances the baby dressed and wrapped in a blanket is placed in the mother s bed The mother the infant and the father who has often attended the delivery of the infant stay together in the delivery room until approximately 2 hours after the actual time of birth when they are transferred to the maternity ward

Extra contact immediately following delivery (P+ group)

Mouth and upper airways are rinsed the stomach emptied the body dried with a towel and a numbered bracelet fastened around the wrists of infant and mother as in routine care After clamping of the cord 2–6 min following delivery the midwife puts the naked baby onto the mother s abdomen and the infant s back is covered with a blanket This skin to skin contact begins approximately 10 minutes post partum Some five minutes later the midwife moves the baby upwards onto the mother s chest and helps him to suckle from his mother s breast i e approximately 15 min post partum (Fig 1) This extra contact lasted for about 10 to 15 min After this period when the baby was about 25–30 min old the normal routine procedure as described above was continued

Routine care at the maternity ward from about 2 hours after delivery until discharge from hospital 6–8 days later (P+ P and M groups)

During the first three days the mother sees and nurses her infant every 4 hours during the day The infant stays during the night and most of the day in a separate baby room During the second half of the post partum week the infant stays during the daytime in his mother s room The mother takes a more active part in the care of her infant giving him a bath changing nappies and clothes and so on In most rooms there is accommodation for four mothers and their infants

Observations of mother and infant behaviour

Before leaving the delivery floor the mothers were asked to participate with their infants in an ongoing study and told that observation was going to take place later on in the maternity ward None of the mothers refused probably because the approach was via the midwives who by that time were well known to and trusted by the mothers Observation of all subjects was made about 36 hours after delivery (range 32–40 hours) in the mother's own room during breast feeding Two observers participated in the study and the subjects were randomly assigned to one of them They did not know to which group the mother–infant pairs belonged Only one observer was present at each observation All mothers were in a 4-bed room During the observation the other mothers in this room were nursing their own babies as usual The position of the mother her infant and the observer is shown in Fig 2 The observer was present in the room a few minutes before the infant was brought in so as to be less obtrusive Observation started immediately the infant s crib was brought into the mother's room No conversation between mother and observer was allowed during the observation Thirty-five different behavioural items[1] were scored and noted on a check sheet

The observation period was 15 min divided into 20 periods of 15 sec actual observation and 20 periods of 30 sec for writing down on the check-sheet all that had happened during the previous 15-sec period A small tape recorder provided signals through an ear microphone to indicate the observation and writing periods The use of this apparatus was explained to the mothers before the actual observation was started Behaviour that occurred during that period was scored as 1 At the end of the observation the score for every item of behaviour was added up and the total was used as a measure of frequency of the particular behaviour during the observation The maximum score for any item was therefore 20 Behaviour not included in our observation sheet did sometimes occur



Fig 2 The position of the mother the infant and the observer during the observation at 36 hours post partum

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[1] They were defined in co-operation with Mary Anne Trause and based mainly on experience gained in studies performed at the Department of Paediatrics Case Western Reserve University Cleveland USA (Head Marshall H Klaus) Complete data on selection drop-out definitions of the behavioural items the check sheet and detailed results of all observations can be obtained on request from the principal author





Fig 1 a and b The extra skin to skin contact and suckling contact

ling contact 20 primiparae and their infants with the usual routine care after delivery and 20 multiparae and infants with routine care At 36 hours observation was made of maternal and infant behaviour during breast feeding The difference in experimental conditions between Klaus et al and this study is that they studied the impact of early and extended contact, while we only studied the impact of extra contact immediately following delivery (early contact) on maternal behaviour Furthermore we looked at infant behaviour

This paper is the first report of a longitudinal study in which the impact on the mother to infant relationship of this extra contact immediately after delivery will be studied at different times and by means of various techniques such as direct observations medical check ups interviews and studies of infant development

MATERIAL

The study included 62 mother-infant pairs divided into three groups

1 *P+* group Primiparous women (n=22) given Extra contact with their newborn infants followed by Routine Care
2 *P* group Primiparous women (n=20) given Routine Care with their newborn infants
3 *M* group Multiparous women (n=20) given Routine Care with their newborn infants

Selection criteria The basic principle for participation in the study was that mothers and infants should be healthy and live in the Umeå area and that pregnancy and delivery should have been normal The groups were comparable as to mean maternal age social categories education civil state mean number of visits to antenatal clinic and maternal weight gain during pregnancy An equal proportion of the fathers was present at delivery the mean duration of labour and amount of analgesia used were comparable in the two groups of primiparae whereas shorter duration of delivery and less use of analgesia during labour was observed for the *multiparae* The number of breastfeedings before the actual observation and time of observation after delivery did not differ between the three groups Other criteria that all mother-infant pairs had to meet were no history of previous abortions or miscarriages no use of drugs except iron medication and vitamins during pregnancy normal weight gain (14) normal blood pressure and Hb-percentage and no proteinuria (13) The mother should have come into labour spontaneously at fullterm All infants should have been born in vertex presentation and have had no signs of intra or extra uterine asphyxia There had to have been no signs or symptoms of congenital malformation or disease at physical examination 1 day and 6 days post partum Many of these criteria are described by Prechtl et al (15) as optimal obstetrical conditions and others have been used by Thoman et al (18)

Selection procedure The midwives made a preliminary selection of the mothers to study when they arrived at the hospital for delivery and allocated them to one of the above mentioned groups At this point mothers and midwives were not familiar with each other This preliminary selection was thus solely based on existing data concerning previous obstetric history present pregnancy and residence within the Umeå area By this preliminary procedure 74 mother-infant pairs were selected for the study Twelve mother-infant pairs who did not fulfil the established criteria concerning residence delivery infant and neonatal period were excluded The final study groups thus comprised 62 mother-infant pairs

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Table 1 *Maternal and infant behaviour during breast feeding on the second day of life*

Figures denote mean frequency of behaviour during the twenty 15 sec observation periods $P+$=primiparous mothers and infants with extra contact P=primiparous mothers and infants with routine care M=multiparous mothers and infants with routine care

Observation	Mean frequencies			*p* Values		
	P+ (*n*=22)	*P* (*n*=20)	*M* (*n*=20)	*P*+/*P*	*P*+/*M*	*P*/*M*
Mo lying down	8 1	9 2	3 2		0 04	0 02
Mo leaning on elbow	2 4	7 9	3 5	0 02		0 08
Mo sitting up	10 1	3 3	13 6	0 009	0 2	0 003
Infant crying	1 0	2 4	0 5	0 2		0 03
Holds infant	10 9	2 9	11 7	0 001		0 0005
Encompassing	9 3	5 3	10 9	0 1		0 03

Some of this was noted but none of it could be used during data analyzing

Inter observer reliability

The two observers were compared before as well as after the study proper Each of these reliability studies comprised 8 mother–infant pairs The observation was—as in the study—performed during an ordinary breast feed These mothers and infants did not meet any specific criteria other than that pregnancy delivery and the first post partum days had been normal The mothers had agreed to the presence of two observers The correlation coefficient (r) was above 0 90 for all of the 35 behavioural items both before and after the study proper

Statistical methods

For every item observed the mean frequency for each of the three observation groups ($P+$ P and M) was calculated The t test was used in analyzing these results and p values given were obtained by this method $p<0\ 05$ is regarded as significant

RESULTS

1 *Mother–infant behaviour at 36 hours Comparison of groups P+, P and M*

Primiparous mothers with extra contact differed in a highly significant way from those with routine care in three of the 35 items observed two of which were mutually exclusive (Table 1) Multiparous women with routine care differed in only one instance from primiparous women with extra contact but in five from primiparae with routine care The mother s position differed greatly between the three groups during the observation $P+$ and M mothers were sitting up more frequently while P mothers were lying down or leaning on one elbow more often $P+$ and M mothers held heir infants more often than did P mothers Encompassing which could occur both when mothers were sitting and when they were lying down was observed twice as often in $P+$ as in P mothers although this difference was not significant Similarly M mothers were encompassing their infants significantly more than P mothers Infants of P mothers were crying significantly more frequently than infants in the M group and twice as often as $P+$ infants

2 *Mother–infant behaviour by sex of infant within each of the three groups P+ P and M*

The most marked differences were found within the $P+$ group (Table 2) Mothers in this group were holding male infants significantly more often made more attempts to burp their male infants showed more encompassing smiled and looked en face more frequently at boys The $P+$ mothers talked more often to their girls The differences within the P group by sex of infant were much less pronounced significant only for infant on knees and encompassing which was more common in mothers with females Within the M group no prominent differences were seen in maternal or infant behaviour when mothers with males and females were compared

Table 2 *Behaviour differences by sex of infant within the three groups (P+ P and M)*

P+=primiparae with extra contact P=primiparae with routine care M=multiparae with routine care

Observation	P+ group (12♂–10♀) p values	P group (13♂–7♀) p value	M group (10♂–10♀) p value
Burps	0 04	0 2	–
Infant on knees	–	0 05	–
Holds	0 07	0 08	–
Encompassing	0 07	0 01	–
En face	0 04	0 07	–
Talks to infant	0 01	0 08	–
Smiles at infant	0 07	0 2	–

3 *Mother–infant behaviour by care and parity groups P+ P and M within each sex*

Mothers with male infants P+ mothers were *sitting up holding and encompassing* their boys with close body contact significantly more often than P mothers (Table 3) The P+ mothers also made more frequent attempts to let their boys burp P+ infants were significantly less quiet than M infants Like the P+ mothers with male infants M mothers were *sitting up* significantly more often than P mothers male infants in the P group were crying more frequently

Mothers with female infants No significant differences were found for P+ and P mothers and their female infants P+ mothers *with girls* were lying down significantly more often (p=0 006) *and talked less frequently* (p=0 03) to others than M mothers with girls No significant differences *were seen in maternal and infant* behaviour when comparison was made between P and M group with female infants

DISCUSSION

The results of the present study indicate that mothers with boys behave differently from mothers with girls although there has been the same immediate post partum care that differences in behaviour occur at 36 hours post partum between a group of mothers and infants who have had 15–20 min extra skin to skin and suckling contact immediately following delivery (P+) and a group of mothers and infants with routine care (P) that the influence of post partum care is greater on the behaviour of boys and their mothers than on girls and *their mothers Differences in behaviour be*tween primiparous and multiparous mothers and their infants were also found the differences being most pronounced between primis and multips with routine care and very small between primis with extra contact and multips with routine care At an interview with the mothers no differences were found between P+ and P mothers with regard to their relationship with their own mothers their husbands *planning of pregnancy* preparations for pregnancy delivery and baby or for percep

Table 3 *Behaviour mean frequencies and differences by care and parity within the infants of male sex*

P+=primiparae with extra contact P=primiparae with routine care M=multiparae with routine care

Observation	Mean frequencies P+ (n=12)	P (n=13)	M (n=10)	p values P+ vs P p	P+ vs M p	P vs M p
Mo sitting up	15 1	1 9	14 1	0 0009	–	0 0009
Infant quiet	4 7	7 5	9 5	0 1	0 04	–
Infant crying	0 9	2 9	0 2	0 1	–	0 03
Burps	0 9	0 1	1 4	0 04	–	0 2
Holds	14 7	0 5	13 3	0 0001	–	0 001
Encompassing	17 1	2 2	10 7	0 001	–	0 01
Close contact	18 1	13 0	17 5	0 04	–	0 07

tion of pregnancy, delivery and first post partum week It is thus not probable that such background factors could account for the observed differences in behaviour

The findings in $P+$ and P groups are similar to those reported by Klaus et al (4) in a study of 14 mother–infant pairs with and without extended contact The main difference between the two studies is that Klaus et al gave their babies one hour of naked contact following birth and in addition five extra hours with their mothers each afternoon of the three days after delivery while in the present study only 15–20 min of nude extra contact separated the two groups ($P+$ and P) The fact that this brought about the same effect gives emphasis to the opinion that the very first hours after delivery may be of the greatest importance If this is true man will fit into a pattern similar to that of all other mammals studied (7)

Klaus et al combined several behavioural items into groups (attachment behaviour for example) and could then obtain significant differences between care groups The present study extended these findings demonstrating significant differences even for individual behavioural items The fallacies in the methods used in studies of this kind are still incompletely explored and some points will therefore be discussed here Using 35 observational items one can expect some differences on the 5% level to occur by chance although some items excluded others More differences than could be expected by chance were found

Direct observation was preferred to filming although the presence of an observer is bound to have some effect (9) We found however that the observation session can be arranged as a fairly natural part of the care routine and we agree with Costello (3) that the flexibility of the human eye and brain far surpasses that of the camera In addition we found that a high inter observer reliability can be obtained Filming may be experienced as a less natural way of observation and even extensive re examination of film or video tape leaves many episodes ambiguous (3) Film observations are, however, certainly superior where intervals and sequences of behaviour are to be studied A one way screen is a third possible method which however, was not available to us

Differences in maternal behaviour during breast feeding depending upon parity have been reported by Thoman et al (17 18) primiparae spending more time in non feeding activities and changing activities In our study pronounced differences in maternal and infant behaviour were found between multiparae (M) and primiparae (P) primiparae with extra contact ($P+$) and multiparae (M) and their infants were however very much alike This may give further support to the opinion that extra contact influences maternal and infant behaviour

Studying visual alertness in certain neonates Korner & Thoman (8) found no differences between male and female infants but Leidermann et al (10) found differences in maternal behaviour depending on sex of infant Moss (11) found pronounced differences in both maternal and infant behaviour at three weeks boys sleeping less and crying more resulting in more extensive and stimulating interaction with the mother When the state of the infants was controlled most of these sex differences became weaker however The sex differences observed in our investigation thus have some support in earlier studies but extend these by showing that early contact had far more profound effects on boy–mother than on girl–mother pairs In the P+ mothers of boys were holding smiling (1) and making attempts to burp them more often while mothers of girls were talking to them more frequently Within the P group the differences correlated to sex of infant were much smaller mothers with girls having them more on their knees and encompassing Within the M group no significant differences were found

The eventual effects of extra neonatal contact on the relationship between mother and infant cannot be evaluated as yet but since we in a three months follow up study of $P+$ and P still do find differences in maternal and

even more in infant behaviour (2) the possibility exists that the extra post partum contact has a long term impact on the relationship between mother and infant Support for this may also ge given by the fact that early contact mothers ($P+$) breast fed for a longer period (2) In a number of follow-up studies one month (4) one year (5) and two years (16) after delivery Klaus et al also found differences in maternal behaviour between their extended contact group and control group The mere knowledge of the importance and possible positive effects of extra contact shortly after delivery may help to minimize the deleterious influence of necessary separation in cases of prematurity asphyxia malformation and the like A mother who refuses an extra contact offered to her may need to get support and help during her stay in the maternity ward Lastly the mode of neonatal care may be an important factor to take into account in studies of child development

ACKNOWLEDGEMENT

This study was supported by the Medical Faculty University of Umeå Semper and the Åhlén Foundation

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Submitted Febr ?6 1976
Accepted Sept 28 1976

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Submitted Febr 26 1976
Accepted Sept 28 1976

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Acta Pædiatr Scand 66 145–151 1977

LONG TERM EFFECT ON MOTHER–INFANT BEHAVIOUR OF EXTRA CONTACT DURING THE FIRST HOUR POST PARTUM

II A Follow up at Three months

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ABSTRACT de Chateau P and Wiberg B (Department of Paediatrics and Department of Child Psychiatry University of Umeå Umeå Sweden) Long term effect on mother–infant behaviour of extra contact during the first hour post partum II A follow up at three months Acta Paediatr Scand 66 145 1977.—Primiparous mothers and their infants who had an extra 15–20 minutes suckling and skin to skin contact during the first hour after delivery behaved differently at 36 hours post partum compared with a control group without this extra contact The present study is a 3 month follow up of these mothers and infants by means of direct observation of mother–infant free play and a personal interview with the mothers Mothers in the extra contact group spent more time kissing and looking en face at their infants these infants smiled more often and cried less frequently A greater proportion of the mothers with extra contact were still breast feeding at 3 months The influence of extra contact on behaviour was more pronounced in boy–mother than in girl–mother pairs

KEY WORDS Mother–infant behaviour extra contact neonatal period follow up

Primiparous mothers and their infants who had had 15–20 min suckling and skin to skin contact (extra contact) during the first hour after delivery behaved differently at 36 hours post partum compared with a control group without extra contact (4) The differences were much greater for male infants and their mothers than for females

The two primiparous mother–infant groups (P+ and P) those with and those without extra contact have been observed at a home visit 3 months after delivery This follow up also included a personal interview with the mothers covering pregnancy neonatal week and the first 3 months after discharge from the maternity ward The results of the observation studies at 36 hours after delivery were not known to the observers at the time of the 3 month follow up study The results of the observational part will be presented and discussed in this paper in relation to other similar studies Some preliminary results of the personal interview will be given

The aim of the present study was to ascertain whether (*a*) there were any notable differences between infants of different sex with the same type of care and (*b*) whether changes in care had a different influence on female and male infants and their mothers

MATERIAL

All mothers were primiparous The study group mothers (P+) had had 15– 0 min suckling and skin to skin contact (extra contact) with their newborn babies during the first hour after delivery The control mother–infant pairs had had no such contact Otherwise the groups had been cared for in the same way The mother–infant pairs were randomly assigned to one of the two groups before delivery Details of selection and care have been described earlier (4) Background data were comparable in both groups (4) No differences between the two groups were



C

Fig 1 The approximate placement of mother (*A*) and infant (*B*) on the carpet (shaded area) and observer (*C*) during observations of free play at 3 months

found in the number of days the mother–infant had been staying in the maternity ward the mean infant weight loss during the neonatal period the number of contacts with the Child Health Centre the mean infant age at observation or the infant s postnatal growth and psychomotor development In both study group and control group one mother–infant pair participating in the first observation study (4) was lost on the present study One of the mothers was on holiday at the time of follow up and one had left the country for an extended stay abroad

METHODS

After completing the observation at 36 hours in the maternity ward (4) the observer talked to the mother and answered questions During this conversation we asked if we could contact her again for a follow up study when the infant was 3 months old All mothers agreed to this follow up The same two observers as earlier (4) participated in this study The appointments for home visits were made by a secretary The mother–infant pairs were thereafter randomly assigned to one of the two observers neither of them being aware of the group (*P*+ and *P*) to which the mother–infant pair concerned belonged The follow up study was done during a home visit and included a mother–infant free play observation A personal interview was held with the mothers This interview covered mother s perception of pregnancy delivery the neonatal week and first 3 months at home

Time of home visit

All home visits were made at 1 p m At this time most infants were asleep After an initial interview mother–infant free play approximately 2–2½ hours after the last feeding was observed This is a good time for observation (1) since the infant is usually alert and cooperative

Arrangements

The mother sat with the infant on the carpeted floor and the observer also sat on the floor approximately one metre away from them (Fig 1) Before the observation started the mother was told by the observer that we wanted to watch her and her infant during 10 min free play The mother was given a bell a dangling ring and a rattle bag (Fig 2) similar to those used in Gesell s Developmental Test (7) The mother could use the toys in whatever way and sequency she liked

Behavioural items

Sixty one different behavioural items were scored and noted on a check sheet most of which are very descriptive of what was observed [1]

Duration and technique of observation

During a pilot study we discovered that infants very soon got tired during observation The mean frequency for crying during observation of 8 infants was 0 25 during the first 5 min and 1 40 during the second 5 min period Similarly the mothers used a dummy test more frequently during the second half of the observation time than during the first half The observation periods were therefore kept as short as 10 min and good co-operation was obtained from all mother–infant pairs studied The observation period of 10 min was divided into 10 periods of 15 sec actual observation and 10 periods of 45 sec for writing down all that had happened during the previous 15 sec period Behaviour that occurred during that period was scored as 1 At the end of the observation the scores for all items of behaviour were added up and the total was used as a measure of the frequency of that particular behaviour during the observation The maximum score for any item was therefore 10 Behaviour not included on our observation sheet sometimes occurred Some of this was noted but none of it could be used during data analysis A tape recorder provided signals through an ear microphone to indicate the observation and writing periods

The use of this apparatus was known to the mothers from our first observation at the maternity ward (4) and if necessary re explained to the mothers before the observation started

Inter observer reliability

For reliability purposes a study was made of 11 mother–infant pairs during a routine 3 month check up at the

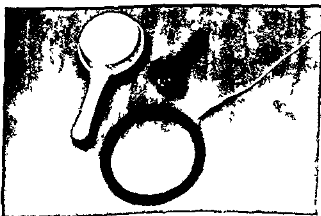


Fig 2 The bell dangling ring and rattle bag used during the observation of mother–infant free play

[1] Complete data on background definitions of the behavioural items the check sheet and the complete results of the observations can be obtained on request from the principal author

Child Health Centre in our hospital before the study proper started These mothers and infants did not meet any specific criteria other than that pregnancy delivery the first postnatal week at the maternity ward and the first 3 months at home had been normal they also agreed to participate in the reliability study and on the presence of two observers For none of the 61 items used during the observation the correlation coefficient (r) was lower than 0 9

Statistical methods

For every item observed the mean frequency for each of the two groups was calculated and the t test used in evaluating the level of significance The p values given are obtained by this method In the reliability study the correlation coefficient r was used

Table 1 *Primiparous mothers without extra contact (P)*

Boys compared with girls Mean frequencies and p-values

Observation	Mean frequency		p value Boys/ girls
	Boys (n=12)	Girls (n=7)	
Infant behaviour			
Eyes closed	0	3 1	0 05
Eyes open	10	6 9	0 02
Plays with toys	5 7	3 1	0 05
Maternal behaviour			
Cleans	0 1	1 1	0 02
Gives toy	4 8	2 7	0 08

RESULTS

A *Observations of maternal and infant behaviour during free play*

The results of observations during the mother–infant free play are shown in Tables 1–5 First a comparison is made within groups with the same type of care but different sex of infant (Tables 1 and 2) Thereafter we present the results for different kind of care and compare for each sex separately (Tables 3 and 4) and finally make a comparison between groups with different post partum care as a grand total not relating to sex of infant (Table 5) Only those items of behaviour with p values in the t test of 0 08 or less are given in the Tables

Primiparous mothers without extra contact (P) comparison of behaviour with regard to sex of infant

Male infants had their eyes open during the entire observation period (Table 1) while female infants had their eyes closed during a part of the time observed (p=0 05) Consequently male infants were more alert and played with one of the three toys used (bell rattle dangling ring) during a greater proportion of the observation time (p=0 05) and mothers gave toys to boys to play with more often (p=0 08) Girls were crying somewhat more frequently and mothers cleaned them significantly more often (p=0 02) than boys

Primiparous mothers with extra contact (P+) comparison of behaviour with regard to sex of infant

Boys and girls were alert with eyes open during the entire observation period (Table 2) however male infants played more frequently with their hands (p=0 04) Mothers in both groups looked at their infants during the entire observation period mothers of boys spent a significantly longer proportion of their time in the en face position (p−0 04)

Primiparous mothers with extra contact (P+) and without extra contact (P) compared by sex of infants Boys

Mothers in the extra contact group (Table 3) looked en face (p=0 01) and kissed their boys significantly more often (p=0 01) they smiled

Table 2 *Primiparous mothers with extra contact (P+)*

Boys compared with girls Mean frequencies and p values

Observation	Mean frequency		p value Boys/ girls
	Boys (n=11)	Girls (n=10)	
Infant behaviour			
Plays with hands	1 5	0 1	0 04
Maternal behaviour			
Looks en face	4 6	1 5	0 04



Fig 1 The approximate placement of mother (*A*) and infant (*B*) on the carpet (shaded area) and observer (*C*) during observations of free play at 3 months

found in the number of days the mother–infant had been staying in the maternity ward the mean infant weight loss during the neonatal period the number of contacts with the Child Health Centre the mean infant age at observation or the infant s postnatal growth and psychomotor development In both study group and control group one mother–infant pair participating in the first observation study (4) was lost on the present study One of the mothers was on holiday at the time of follow up and one had left the country for an extended stay abroad

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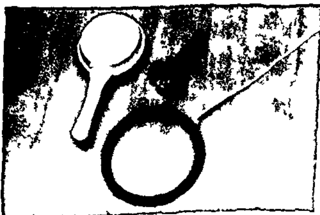


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nity ward and felt that adaptation to their infants was somewhat more difficult More mothers in the extra contact group were still breast feeding their infants at 3 months than in the control group (6) Further details of the personal interview with the mothers will be published later (5)

DISCUSSION

The present study has shown that 3 months after delivery (1) maternal behaviour with boys and girls within groups with the same type of immediate postnatal care differs in a number of ways and (2) that different kinds of neonatal care do influence both maternal and infant behaviour In the extra contact ($P+$) group mothers spent more time looking en face and kissing and their infants laughed or smiled more frequently In the control group (P) mothers spent more time cleaning their infants and these cried significantly more often The effects of the extra contact during the first hour after delivery were more pronounced for boys and their mothers than for girls and their mothers For example mothers in the extra contact group ($P+$) kissed and smiled at their boys more frequently and boys in the extra contact group ($P+$) smiled more often at their mothers than in the P group No such differences between $P+$ and P were found for girls and their mothers Differences in maternal and

Table 5 *All subjects comparison P+ and P*
Mean frequency and p values

Observation	Mean frequency $P+$ ($n=21$)	P ($n=19$)	p value $P+/P$
Infant behaviour			
Crying	0 2	1 2	0 02
Smiling/laughing	2 7	1 4	0 02
Maternal behaviour			
Looks en face	3 1	0 8	0 008
Kisses	1 1	0 3	0 009
Cleans	0 1	0 5	0 05

Table 6 *The personal interview with all mothers*

	P+ ($n=21$)	P ($n=19$)
Pregnancy		
Planned and welcome	12	12
Unplanned and welcome or not welcome	8	5
Unplanned and not welcome	1	2
Participation in antenatal programme	17	12
Preparation for delivery		
Little	11	8
Moderate	2	6
Much	8	5
Perception of delivery		
Difficult	4	1
Normal	3	3
Easy	14	15
Husband s visit to mat ward		
Frequent	16	16
Not frequent	4	2
First week at home		
Difficult	19	15
Easy	2	3
Adaptation to child		
Easy	13	3
As expected	7	14
Difficult	1	2
Problems with night feeding		
Yes	1	6
No	17	10
Mean time of night feeding (in days)	42	24
Mean number of days the mother had help at home	7 6	19 5
Percentages of mothers still breast feeding	58	6

infant behaviour between the two groups extra contact versus routine care were also found during an earlier observation at 36 hours after delivery (4)

Background factors concerning pregnancy delivery neonatal period (4) and the first 3 months at home (Table 6) were comparable in the two groups thus reinforcing the reality of the observed differences in behaviour The methodology in this field is so far incompletely explored Advantages and disadvantages of the methods used here were discussed in a previous paper (4) and it was concluded that reliable results can be obtained Using 61 observational items many of which could not appear simultaneously some chance differences in behaviour significant at the 5% level can be

Table 3 *Primiparous mothers with (P+) and without extra contact (p)*
Boys Mean frequencies and *p* values

Observation	Mean frequency P+ (n=11)	P (n=12)	p value P+/P
Infant behaviour			
Smiling/laughing	3 0	1 3	0 03
Maternal behaviour			
Looks en face	4 6	0 8	0 01
Smiles	5 9	3 7	0 07
Kisses	1 2	0 3	0 01
Others present	4 8	1 5	0 08

somewhat more frequently at their boys ($p=0\ 07$) and other persons (father visitor) were slightly more often present during our observation ($p=0\ 08$) Boys with extra contact smiled significantly more frequently at their mothers ($p=0\ 03$) though no differences were found in alertness (eyes open, eyes closed)

Primiparous mothers with extra contact (P+) and without extra contact (P) compared by sex of infant Girls

Maternal behaviour (Table 4) differed significantly in only one respect mothers without extra contact cleaned (with a napkin or a piece of cleaning tissue) their girls more often ($p=0\ 02$) Girls in the extra contact groups had their eyes open during the entire period of observation whereas girls in the non contact group had their eyes closed ($p=0\ 08$) for some part of the observation period The *P* girls also cried slightly more frequently ($p=0\ 09$)

Primiparous mothers with (P+) and without extra contact (P)

A number of significant differences (Table 5) in maternal behaviour were found mothers in the extra contact group spent more time in looking en face ($p=0\ 008$) and kissing their infants ($p=0\ 009$) while they less frequently cleaned their infants ($p=0\ 05$) Holding the infant was equally frequent in both groups but mothers with extra contact more frequently cuddled their infants though this difference was not significant Infant crying was more frequently observed for non extra contact infants ($p=0\ 02$) and co variated with mother rocking more frequently the difference however did not reach a significant level Infant smiling and/or laughing appeared significantly more often in the extra contact infants ($p=0\ 02$)

B *The personal interview with the mothers*

The main results are shown in Table 6 The groups were comparable as regards planning of pregnancy and whether this was welcome or not the mother s perception of physical well being during the first 4 months and the second part of pregnancy participation in antenatal clinic programmes preparations for and perception of delivery husband s visiting patterns at maternity ward and mother s perception of first week at home The infants in both groups slept equally long at 3 months in both groups and an equal number had suffered from colic and received medication for it Although the mothers reported the same frequency of waking at night and infants in the extra contact group were given night feeds twice as long as infants in the control group mothers in the control group reported more problems with night feeding Mothers in the control group had help in the household during a longer period after discharge from the mater

Table 4 *Primiparous mothers with (P+) and without extra contact (P)*
Girls Mean frequencies and *p* values

Observation	Mean frequency P+ (n=10)	P (n=7)	p value P+/P
Infant behaviour			
Eyes closed	0	2 9	0 08
Eyes open	10 0	7 1	0 04
Maternal behaviour			
Cleans	0 1	1 1	0 02

nity ward and felt that adaptation to their infants was somewhat more difficult More mothers in the extra contact group were still breast feeding their infants at 3 months than in the control group (6) Further details of the personal interview with the mothers will be published later (5)

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Mean frequency and *p* values

Observation	Mean frequency *P*+ (*n*=21)	Mean frequency *P* (*n*=19)	*p* value *P*+/*P*
Infant behaviour			
Crying	0 2	1 2	0 0?
Smiling/laughing	7	1 4	0 0?
Maternal behaviour			
Looks en face	3 1	0.8	0 008
Kisses	1 1	0 3	0 009
Cleans	0 1	0 5	0 05

Table 6 *The personal interview with all mothers*

	P+ (*n*=21)	P (*n*=19)
Pregnancy		
Planned and welcome	12	12
Unplanned and welcome or not welcome	8	5
Unplanned and not welcome	1	2
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Preparation for delivery		
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Easy	2	3
Adaptation to child		
Easy	13	3
As expected	7	14
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Problems with night feeding		
Yes	1	6
No	17	10
Mean time of night feeding (in days)	42	24
Mean number of days the mother had help at home	7 6	19 5
Percentages of mothers still breast feeding	58	26

infant behaviour between the two groups extra contact versus routine care were also found during an earlier observation at 36 hours after delivery (4)

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Observation	Mean frequency *P+* (*n*=11)	*P* (*n*=12)	*p* value *P+/P*
Infant behaviour			
Smiling/laughing	3 0	1 3	0 03
Maternal behaviour			
Looks en face	4 6	0 8	0 01
Smiles	5 9	3 7	0 07
Kisses	1 2	0 3	0 01
Others present	4 8	1 5	0 08

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Table 4 *Primiparous mothers with (P+) and without extra contact (P)*
Girls Mean frequencies and *p* values

Observation	Mean frequency *P+* (*n*=10)	*P* (*n*=7)	*p* value *P+/P*
Infant behaviour			
Eyes closed	0	2 9	0 08
Eyes open	10 0	7 1	0 04
Maternal behaviour			
Cleans	0 1	1 1	0 02

maternal responsivity (11) or to a biological mechanism for example differences in signals given by boys and girls must await further studies Moss (13) found differences in behaviour of mothers with boys and girls reared under similar circumstances The change in post partum care seems to reinforce this already pre existing difference between the two sexes

ACKNOWLEDGEMENT

This study was supported by the Medical Faculty University of Umeå Semper and the Åhlen Foundation

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expected The differences in maternal and infant behaviour in the present study are however more pronounced than can be expected by chance only

Klaus et al (8 9 15) have shown more systematically than others that neonatal care influences maternal behaviour both in the neonatal period and later on The present study extends their observations in three respects First it suggests that a naked 15–20 min extra contact was associated with changes in maternal behaviour similar to those accompanying the considerably longer extra contact used by Klaus et al i e one hour immediately after delivery and an additional 5 extra hours each afternoon during the next 3 days Secondly Klaus found differences between the treatment groups only when he lumped together several behavioural items as what he called maternal attachment behaviour' In this study we found differences in separate behavioural items In addition to maternal behaviours which can be involved in development of attachment such as kissing looking en face smiling and crying (3) we found differences also in other behaviour such as cleaning of infant and rocking Thirdly we found differences in infant behaviour which have not been studied by Klaus et al (8 9 15)

In our view many of the differences in behaviour observed have an emotional background and a value in the relationship between mother and infant Behaviour which can be expected to have a positive influence and value was found to occur more frequently in the extra contact group (*P*+) One of the marked differences in infant behaviour between the two groups concerned crying and smiling The former occurred more frequently in the routine care group the latter more frequently in the extra contact group Infant crying could be a sign of less well developed mother–infant synchrony It elicited cleaning and rocking more often in mothers in the control group Cleaning as well as rocking (2) may be interpreted as a kind of maternal soothing behaviour Such behaviour was more frequent in mothers with routine care and this could be the effect of more frequent infant crying in this group Infant smiling and laughing on the other hand was more frequent in the extra contact group possibly acting as a release mechanism eliciting instinctual responses in the mother (2) and serving as an activator of positive maternal behaviour mothers in the extra contact group more frequently showed such positive maternal behaviour i e kissing and regarding en face These findings receive support from Lewis (11) who during observation of mothers and 3 month old infants found a positive correlation between mother s smiling and infant s smiling We will not try to evaluate the impact of differing behaviour on later development only to mention that Bowlby (2 3) and Richards (14) agree that smiling plays a role in the growing mother–infant relationship

In studies on monkeys Mitchell (12) found that frequency and form of mother–infant contact depends on the behaviour of the mother and on the age and sex of infant In the present study we found differences in maternal and infant behaviour within groups with the same care depending on the infant s sex For example in the *P*+ group mothers with boys looked en face more frequently in the *P* group mothers cleaned girls more often Such sex differences might be acquired caused by difference in maternal attitudes associated with difference in their expectations of boys and girls Obviously they might also have a more biological background The fact that sex differences were already present at 36 hours (4) might speak in favour of a genetic cause or at least a prenatal moulding A most interesting and unexpected finding was that a change in care routine influenced boys and their mothers more than girls and their mothers For example infant smiling was seen more frequently in the *P*+ group both for girls and boys the influence being greatest on the boys however (Tables 3 and 4) Not only did infants behave differently in relation to sex but so also did mothers i e in kissing although whether this was due to acquired difference in attitude to

maternal responsivity (11) or to a biological mechanism for example differences in signals given by boys and girls must await further studies Moss (13) found differences in behaviour of mothers with boys and girls reared under similar circumstances The change in post partum care seems to reinforce this already pre existing difference between the two sexes

ACKNOWLEDGEMENT

This study was supported by the Medical Faculty University of Umeå Semper and the Åhlen Foundation

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Submitted Febr 6 1976
Accepted Sept 28 1976

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EFFECTS OF GROWTH HORMONE ON PROTEIN METABOLISM

Acute Changes in Plasma Amino Acids in Growth Retarded Patients with and without Growth Hormone Deficiency

NIKOLAUS STAHNKE CHRISTA PLETTNER and WERNER BLUNCK[1]

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ABSTRACT Stahnke N Plettner Ch and Blunck W (Department of Paediatrics University Hospital Hamburg and Children s Hospital Altona Hamburg West Germany) Effects of growth hormone on protein metabolism Acute changes in plasma amino acids in growth retarded patients with and without growth hormone deficiency Acta Paediatr Scand 66 153 1977.—39 patients with growth retardation were investigated 21 (group H) were suffering from GH deficiency and 18 (group N) had no endocrine disease except for two adequately treated patients with mild hypothyroidism Analysis of 15 plasma amino acid concentrations was carried out before and 1 and 2 hours after intravenous HGH *injection* at a dosage of 2 mg per m Except for one amino acid no significant difference between mean pre treatment amino acid values was observed in the two groups of patients In group H there was a highly significant decrease in plasma concentration of 14 amino acids already 1 hour after HGH injection and of all 15 amino acids after 2 hours This response of plasma amino acids to HGH was less pronounced in group N For 5 amino acids a moderate correlation was found in group H between acute metabolic response to HGH and growth response to long term HGH treatment Our results following HGH injection may reflect increased plasma amino acid transfer into cells due to HGH

KEY WORDS Growth hormone amino acids hypopituitarism dwarfism

Since Beck (2) 1957 initially recorded significant and consistent metabolic effects of human growth hormone (HGH) in man contradictory actions of HGH have been reported on protein metabolism Following several days of intramuscular HGH administration a marked decrease in blood urea and urinary nitrogen excretion was demonstrated by several studies (7 8 13 14 22 25 36 38) A significant increase in serum alpha amino nitrogen was found in hypopituitary patients after intramuscular HGH treatment for 5–6 days (25) and nearly all investigated amino acids showed an increase in plasma concentration due to 3–5 days of intramuscular HGH administration to hypopituitary dwarfs (40) Contrary to these findings other authors reported a decrease in plasma alpha amino nitrogen in GH-deficient dwarfs following an intravenous injection or infusion of HGH (10 27) In this context it appeared worthwhile to investigate plasma amino acid concentration in growth retarded patients with and without growth hormone deficiency before and after intravenous HGH administration

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MATERIALS AND METHODS

39 patients with growth retardation were examined All patients were below the 3rd percentile for height To evaluate GH secretion plasma HGH was determined during insulin induced hypoglycemia and arginine infusion

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Table 1 *Significant decrease in 8 out of 15 investigated amino acid concentrations in patients without GH deficiency*

Plasma amino acid levels initial (h_0) 1 hour (h_1) and 2 hours (h_2) after HGH administration Sample size is shown in parenthesis p=significance level of differences in means n s =not significant

Amino acid	Plasma concentration (mg/100 ml) (mean±S E M)			p	
	h_0	h_1	h_2	h_0/h_1	h_0/h_2
Threonine	1 74±0 20 (18)	1 71±0 23 (14)	1 58±0 17 (17)	<0 05	<0 01
Alanine	3 23±0 32 (18)	3 00±0 32 (14)	2 67±0 25 (16)	0 01	0 01
Valine	2 38±0 17 (18)	2 34±0 18 (13)	2 22±0 15 (17)	n s	<0 05
Methionine	0 39±0 03 (17)	0 34±0 04 (13)	0 33±0 02 (15)	n s	<0 05
Isoleucine	0 90±0 07 (18)	0 83±0 08 (14)	0 83±0 06 (17)	0 001	<0 05
Leucine	1 38±0 09 (18)	1 32±0 10 (14)	1 26±0 08 (17)	<0 05	<0 05
Tyrosine	0 83±0 06 (18)	0 81±0 06 (14)	0 73±0 05 (17)	n s	<0 05
Phenylalanine	0 92±0 07 (17)	0 79±0 05 (14)	0 76±0 05 (16)	n s	<0 05

(20) Thyroid stimulating hormone function was evaluated by protein bound iodine (PBI) concentration and by thyroid uptake of [131]I ACTH function was evaluated by the 2-day metyrapone test (5) and an ACTH infusion test (37) In response to oral administration of metyrapone the excretion of 17-OH-corticosteroids was measured in urine (31) tetrahydrodesoxycortisol and tetrahydrocortisol being measured separately (5) Plasma corticosteroids were determined after intravenous infusion of corticotropin (4) As a rough screening test for adiuretin deficiency the specific gravity was repeatedly measured in the first urine portion in the morning

21 patients showed clinical and biochemical evidence of hypopituitarism (group H aged 6–21 years 18 males and 3 females) The mean maximum GH response to insulin induced hypoglycemia was 1 27 ng/ml±0 19 (S E M) and to arginine infusion 1 34 ng/ml±0 20 (S E M) 13 of these 21 patients were considered to suffer from isolated GH deficiency However the definite diagnosis of an isolated GH deficiency must await the appearance of puberty in these patients Onset of puberty was observed in 7 of these 13 patients whereas in the remaining 6 patients skeletal maturation was too much retarded to expect pubertal development All studies in this group of patients have been performed prior to HGH treatment

GH deficiency could be excluded in the remaining 18 patients with short stature (group N aged 5–17 years 14 males and 4 females) The mean maximum GH response to insulin induced hypoglycemia was 14 62 ng/ml±2 12 (S E M) and to arginine infusion was 13 04 ng/ml±2 41 (S E M) In two patients a mild hypothyroidism was found they had received adequate thyroid hormone replacement therapy for several months before our investigation started In the remaining 16 patients detailed physical and laboratory evaluation revealed no endocrine disease This group included 7 patients with constitutional delay in growth and sexual maturation 4 patients with familial short stature 4 patients with familial short stature combined with constitutional delay in growth and sexual maturation and 1 patient with Russell Silver syndrome

After an overnight fast patients were kept in bed and were permitted to drink only water At 8 a m they received HGH intravenously at a dosage of 2 mg/m² of body surface area HGH was commercially obtained and prepared by the method of Roos with a potency of 2 international units per mg (Crescormon Deutsche Kabi GmbH Munchen West Germany) Prior to and one and two hours after HGH injection blood was obtained for determination of concentration of 15 amino acids

GH was determined by double antibody radioimmunoassay (26) The lower limit of sensitivity of this assay in our laboratory is 0 5 ng/ml In our laboratory a peak plasma HGH concentration of at least 5 ng/ml is considered a normal GH response (34) Amino acid determinations were carried out by ion-exchange chromatography with a Beckman Unichrom amino acid autoanalyzer according to the procedure described by Spackman et al (33)

Statistical evaluation

Bartlett s test for homogeneity of variance and David s test for normality (ratio of range to standard deviation in the same normal sample) were performed prior to tests concerning the difference of two means According to these results nonparametric tests (the Mann Whitney U test for independent samples and the Wilcoxon sign rank test for correlated data) or the two tailed t test were carried out Group differences (group H and group N see above) in the metabolic response to HGH were compared by analyses of variance Additionally Spearman s coefficient of rank correlation (r) was calculated for the acute metabolic response to HGH injection and the growth response to long term HGH treatment (29) Since all results of amino acid analyses were not available in every patient sample size of data was different

RESULTS

Pre treatment amino acid concentrations in group H compared to those in group N

There was no significant difference between the mean pre treatment amino acid values in group H and group N except for one amino

Table 2 *Significant decrease in all 15 investigated amino acid concentrations in GH lacking patients*

Plasma amino acid levels initial (h_0), 1 hour (h) and 2 hours (h) after HGH administration. Sample size is shown in parenthesis. p = significance level of differences in means. n.s. = not significant

	Plasma concentration (mg/100 ml) (mean±S.E.M.)			p	
Amino acid	h	h	h_2	h_0/h	h_0/h_2
Taurine	*1.68±0.1[illegible] (21)*	1.47±0.10 (19)	1.00±0.13 (16)	n.s.	<0.001
Aspartic acid	0.45±0.04 (15)	0.30±0.03 (13)	0.[illegible]2±0.03 (12)	<0.001	0.001
Threonine	1.77±0.14 (21)	1.46±0.14 (19)	1.29±0.15 (16)	<0.001	<0.001
Serine	1.84±0.12 (21)	1.51±0.11 (19)	1.34±0.11 (16)	<0.001	<0.001
Asparagine/ glutamine	10.6[illegible]±0.63 (21)	9.[illegible]±0.54 (19)	8.95±0.70 (16)	<0.01	<0.01
Proline	*2.14±0.18 (13)*	*1.84±0.18 (13)*	1.56±0.14 (10)	<0.001	0.01
Glutamic acid	1.39±0.1[illegible] (21)	1.16±0.09 (19)	0.96+0.16 (16)	<0.05	<0.05
Glycine	*1.99±0.14 (21)*	*1.64±0.11 (19)*	1.43±0.14 (16)	<0.001	<0.001
Alanine	3.49±0.30 (21)	[illegible].78±0.24 (19)	2.61±0.[illegible]9 (16)	<0.001	<0.001
Valine	*[illegible].71±0.10 (21)*	*[illegible].43±0.1[illegible] (19)*	2.24+0.11 (16)	<0.01	<0.001
Methionine	0.45±0.0[illegible] (19)	*0.35±0.0[illegible] (17)*	*0.27±0.02 (14)*	<0.001	<0.001
Isoleucine	0.94±0.04 (21)	0.8[illegible]±0.05 (19)	0.72±0.04 (16)	<0.01	<0.001
Leucine	1.66±0.07 (21)	*1.41±0.09 (19)*	*1.19±0.09 (16)*	<0.01	<0.001
Tyrosine	1.03±0.08 (18)	0.83±0.07 (17)	0.75+0.08 (14)	<0.001	<0.001
Phenylalanine	0.95±0.05 (18)	0.81±0.04 (17)	0.68±0.05 (15)	0.01	<0.001

acid. The mean pre-treatment concentration of leucine was significantly higher ($p<0.05$) in patients lacking GH than in patients without GH deficiency.

Changes in plasma concentrations of amino acids in group H and group N with HGH therapy

In patients without GH deficiency (group N) there was a significant decrease from the initial level in 8 out of 15 amino acid concentrations 2 hours after HGH injection. 4 of these 8 amino acids showed significantly reduced values already one hour after HGH injection (Table 1). In GH lacking patients (group H) there was a highly significant fall from the initial level in all of the 15 amino acid concentrations 2 hours after HGH injection; all amino acids except for one exhibited significantly decreased plasma concentrations already one hour after HGH administration (Table 2). Summarizing, patients with GH deficiency had a greater and more consistent decrease in amino acid concentration following HGH injection. The two groups of patients (group H and N) showed a significantly different response of 9 plasma amino acids to HGH administration (Table 3).

However, there was some overlap of individual values. Fig. 1 shows the percentage change in individual plasma concentrations of 1 amino acid (methionine) with the smallest overlap.

Relation between acute metabolic response to HGH and growth response to long term HGH treatment

Only for 5 amino acids a moderate correlation was observed in GH lacking patients between

Table 3 *Significant difference in the response of 9 plasma amino acids to HGH administration in patients with (group H) and without GH deficiency (group N)*

p = significant level

Amino acid	p
Taurine	<0.05
Aspartic acid	<0.05
Threonine	<0.025
Asparagine/Glutamine	<0.05
Glycine	<0.05
Methionine	<0.001
Isoleucine	<0.0.5
Leucine	<0.01
Tyrosine	<0.05

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amino acid concentration should be expected (11 39) Two studies have shown that continued fasting does not lead to any consistent and appreciable change in blood amino acid concentration during a period of the same length as in our study (1 11)

Our findings of reduced plasma amino acid levels following HGH administration are probably not due to changed amino acid absorption or excretion since absorption and fecal excretion of nitrogen showed little or no response to HGH administration (22 36 38) No significant influence of GH on urinary excretion and clearance of amino acids was observed after 3–5 days of HGH therapy (40) at high dosage a renotropic effect could be demonstrated earliest on the 4th day (3) All these results however have been obtained in studies performed under conditions not completely comparable to ours

In rats lower serum and higher tissue levels of alpha amino isobutyric acid (AIB) were observed as early as 15–30 min after intraperitoneally GH injection (28) These findings suggest GH stimulation of plasma amino acid transport into tissues and could explain our results following intravenous GH treatment It is well known that GH increases in vitro and in vivo the rate of net transport of amino acids across cell membranes into the intracellular fluid of a variety of tissues (17 23 32 35) These effects have been shown to occur within 10–30 minutes following GH administration (15 17 28) An increase in protein biosynthesis in cells known to occur in response to GH is not only due to enhanced amino acid influx Above all GH directly exerts its protein anabolic action by stimulating ribosomal activity and synthesis of messenger and ribosomal RNA (12 16 35) Whether somatomedin plays an important role in mediating these effects of GH is still open to question (6 18 19) Whereas the reduction in plasma amino acid concentration one and two hours after intravenous injection of HGH most likely reflects increased transfer of plasma amino acids to intracellular compartments the observed increase in serum alpha amino nitrogen (25) and plasma amino acids (40) following several days of intramuscular HGH treatment could be due to stimulation of protein synthesis

Since a clear separation was not possible between patients with and without GH deficiency due to overlap of individual plasma amino acid levels the differential diagnostic value of this short term metabolic test is limited

Only for some amino acids a moderate correlation was observed in GH lacking patients between decrease in amino acid concentration one hour after HGH injection and growth velocity during 6 or 12 months of HGH treatment In a small group of patients the degree of GH induced nitrogen retention was related to the subsequent course of long term HGH therapy (22) In other studies however acute metabolic responses to HGH did not correlate well with height acceleration obtained on long term HGH treatment (8 13 38)

ACKNOWLEDGEMENTS

Skilful technical assistance was rendered by Mrs A Mohrmann In addition the authors wish to thank Drs C Steinhausen and K R Held in helping to prepare this manuscript

This work was supported by the Deutsche Forschungsgemeinschaft Sonderforschungsbereich SFB 34 Endokrinologie

Some findings of this study were presented at the 19 Symposion Deutsche Gesellschaft fur Endokrinologie 1973 Berlin West Germany

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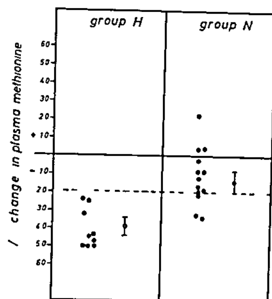


Fig 1 Percentage change in plasma methionine concentration 2 hours after i v HGH injection (individual levels mean±S E M) Group H=GH lacking patients Group N=patients without GH-deficiency

fall in amino acid concentration one hour after HGH injection and growth velocity during 6 months or 12 months of HGH therapy (Table 4)

DISCUSSION

In our study no significant difference was observed between mean pre treatment plasma amino acid concentrations in patients with and without GH deficiency with the exception of one amino acid Lower values for plasma amino acids in hypopituitary patients have been described (24 40) However in a subsequent study of one of the authors (41) two of these amino acids showed higher basal levels in GH lacking patients compared to normal controls Likewise other authors reported nonuniform results Serum alpha amino nitrogen levels were found to be in the same range in hypopituitary patients and in controls (25) In animals hypophysectomy did not change plasma concentration of some investigated amino acids (32), whereas in an other study a marked increase in serum level of alpha aminoisobutyric acid (AIB) was observed (28) For interpretation of all these results the normal circadian fluctuations of blood amino acid levels must be taken into account

In our patients HGH administration led to marked decrease in plasma amino acid concentrations one and especially two hours after HGH injection A much greater responsiveness to exogenous HGH was observed in our patients lacking endogenous GH than in patients without GH deficiency a fact which is well known (7 8 22, 25 36 38 41)

Our findings are in agreement with studies in animals and man A rapid fall in plasma amino acids free amino acid plasma nitrogen and plasma alpha aminoisobutyric acid (AIB) respectively was demonstrated in hypophysectomized animals following GH injection (9 21, 28) In man HGH infusion or intravenous injection cause a marked decrease in serum alpha amino nitrogen levels (10 27) A reduction of two plasma amino acids following intravenously administered HGH has been reported in patients with GH deficiency (41) A significant drop of 4 plasma amino acids after intravenous HGH injection in normal subjects has been described (30)

It is very unlikely that the observed decrease in plasma concentrations of amino acids in our study merely represents circadian fluctuation since for the time under study an increase rather than a decrease of plasma

Table 4 *Significant relation between percentage fall of amino acid concentration 1 hour after HGH administration and growth velocity during 6 months or 12 months of HGH treatment*

r =Spearman s coefficient of rank correlation *p*=significance level of *r* n s =not significant

Amino acid	6 months *r*	6 months *p*	12 months *r*	12 months *p*
Taurine	0 453	<0 05	0 609	<0 025
Glycine	0 493	<0 05	0 593	<0 025
Valine	0 502	<0 05	0 408	n s
Tyrosine	0 489	<0 05	0 374	n s
Phenylalanine	0 478	0 05	0 219	n s

amino acid concentration should be expected (11 39) Two studies have shown that continued fasting does not lead to any consistent and appreciable change in blood amino acid concentration during a period of the same length as in our study (1 11)

Our findings of reduced plasma amino acid levels following HGH administration are probably not due to changed amino acid absorption or excretion since absorption and fecal excretion of nitrogen showed little or no response to HGH administration (22 36 38) No significant influence of GH on urinary excretion and clearance of amino acids was observed after 3–5 days of HGH therapy (40) at high dosage a renotropic effect could be demonstrated earliest on the 4th day (3) All these results however have been obtained in studies performed under conditions not completely comparable to ours

In rats lower serum and higher tissue levels of alpha amino isobutyric acid (AIB) were observed as early as 15–30 min after intraperitoneally GH injection (28) These findings suggest GH stimulation of plasma amino acid transport into tissues and could explain our results following intravenous GH treatment It is well known that GH increases in vitro and in vivo the rate of net transport of amino acids across cell membranes into the intracellular fluid of a variety of tissues (17 23 32 35) These effects have been shown to occur within 10–30 minutes following GH administration (15 17 28) An increase in protein biosynthesis in cells known to occur in response to GH is not only due to enhanced amino acid influx Above all GH directly exerts its protein anabolic action by stimulating ribosomal activity and synthesis of messenger and ribosomal RNA (12 16 35) Whether somatomedin plays an important role in mediating these effects of GH is still open to question (6 18 19) Whereas the reduction in plasma amino acid concentration one and two hours after intravenous injection of HGH most likely reflects increased transfer of plasma amino acids to intracellular compartments the observed increase in serum alpha amino nitrogen (25) and plasma amino acids (40) following several days of intramuscular HGH treatment could be due to stimulation of protein synthesis

Since a clear separation was not possible between patients with and without GH deficiency due to overlap of individual plasma amino acid levels the differential diagnostic value of this short term metabolic test is limited

Only for some amino acids a moderate correlation was observed in GH lacking patients between decrease in amino acid concentration one hour after HGH injection and growth velocity during 6 or 12 months of HGH treatment In a small group of patients the degree of GH induced nitrogen retention was related to the subsequent course of long term HGH therapy (22) In other studies however acute metabolic responses to HGH did not correlate well with height acceleration obtained on long term HGH treatment (8 13 38)

ACKNOWLEDGEMENTS

Skilful technical assistance was rendered by Mrs A Mohrmann In addition the authors wish to thank Drs C Steinhausen and K R Held in helping to prepare this manuscript

This work was supported by the Deutsche Forschungsgemeinschaft Sonderforschungsbereich SFB 34 Endokrinologie

Some findings of this study were presented at the 19 Symposion Deutsche Gesellschaft fur Endokrinologie 1973 Berlin West Germany

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Submitted June 23 1976
Accepted Oct 1 1976

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Acta Pædiatr Scand 66 161–167 1977

ESTROGEN TREATMENT IN TALL GIRLS

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ABSTRACT Kuhn N Blunck W Stahnke N Wiebel J and Willig R P (University Children s Hospital Hamburg West Germany) Estrogen treatment in tall girls Acta Paediatr Scand 66 161 1977.—36 tall girls aged 10.3 to 15 5 years were treated with ethinylestradiol (0 5 mg/day) and norethisterone (15 mg/day) To date treatment for seventeen girls has been completed Before treatment their bone age ranged from 11 0 to 13 5 years and mean height prediction was 185 19 cm (181 40–193 00 cm) After about two years of treatment their final height averaged 178 96 cm (171 60–183 50 cm) Predicted height was reduced by an average of 6 23 cm The reduction in final height was significantly greater when therapy was started before a bone age of 12 years Thirteen girls re-examined after discontinuation of therapy all had regular menstrual bleeding within six months There were no serious or irreversible side effects during the period of observation

KEY WORDS Tall girls estrogen therapy

Since excessive height may cause severe psycho-social problems there is some justification for limiting height in girls by estrogen therapy (11 12 18 23 28 35) Further deterioration of deformities of the spine in growing girls such as scoliosis or Scheuermann s disease can also be ameliorated by this treatment Goldzieher (12) in 1956 was the first to report on a premature halt in growth in girls induced by high doses of estrogens Since then many other reports on this treatment have been published (2 4 5 10 11 13 18 22 23 24 26 28 29 30 31 32 33 35) However there is still no unanimity on an optimal scheme of therapy Since 1971 we have been treating young girls with high doses of estrogens to prevent excessive height or for orthopaedic reasons The results and our general experience of this treatment are reported here

METHOD AND CLINICAL MATERIAL

35 healthy girls and one further girl with Marfan syndrome aged between 10 3 and 15 5 years were treated with high doses of estrogens to prevent excessive height Seven of these girls had their menarche before therapy was started Treatment of seventeen girls has been completed There was no significant difference with regard to chronological age bone age or height during the initial period of observation between these girls and the other nineteen still under treatment Therapy was started only if a final height of more than 185 cm was expected and if first signs of pubertal development were present to avoid the psychological stress of the sudden onset of pubertal development in a young girl (24 35) In a few cases severe psychological problems were the reason for beginning treatment although growth prognosis was less than stated above The growth prognosis was calculated according to the tables by Bayley & Pinneau (1) based on actual height and skeletal maturation as determined from the atlas of Greulich & Pyle (16) The parents and patients gave their written consent after being informed about treatment and its possible side effects The patients were examined at least every six months during treatment which was con

Table 1 *Over all results for patients with completed treatment (n=17)*

	Chron age (y)	Bone age (y)	Height (cm)	Duration of therapy (y)	Final height (cm)	Growth prognosis (cm)	Growth reduction (cm)
X	12 70	12 58	175 08	1 96	178 96	185 19	6 23
Min	10 32	11 00	169 50	1 00	171 60	181 40	1 00
Max	15 50	13 50	179 40	3 32	183 50	193 00	11 80
S D	1 17	0 62	3 02	0 74	3 14	3 29	2 82

tinued until the skeletal age was 15 years or more At first we used mestranol in a few cases but subsequently ethinylestradiol (Lynoral® Progynon M® 0 5 mg/day) for the first 25 days of each cycle and norethisterone (Primolut N® 15 mg/day) for days 21 to 25 were given This was followed by five days without treatment (days 26–30) 13 girls had follow up examinations for 1 5 years after therapy had been stopped

Statistical evaluation was based on the nonparametric Mann Whitney *u* test (7)

RESULTS

Treatment of seventeen girls has been completed At the beginning their mean chronological age was 12 7 years their mean bone age was 12 58 years and their mean height 175 08 cm Their mean predicted height was 185 19 cm After about two years of treatment their mean height was 178 96 cm 6 23 cm less than the predicted height Data (mean range and standards deviation) are presented in Table 1

Where therapy was started before a bone age of 12 years 8 92 cm were saved as compared to 5 49 cm when therapy was started with a bone age between 12 and 13 years This difference is statistically significant ($p<0.02$) In one girl with a bone age of 13 5 years growth prognosis could be reduced by only 1 cm (Table 2)

There was no significant difference in the height finally achieved whether the start of therapy came before or after menarche In the former case 6 43 cm were saved in the latter 5 76 cm (Table 3)

During the first phase of estrogen therapy skeletal maturation advanced more rapidly than the chronological age Later both advanced at the same speed (Fig 1) Growth velocity was reduced to about 3 cm/year during the first six months of therapy to about 2 cm/year during the second six months of therapy and in the following period to about

Table 2 *Results in relation to bone age at start of therapy*

	Chron age (y)	Bone age (y)	Height (cm)	Duration of therapy (y)	Final height (cm)	Growth prognosis (cm)	Growth reduction (cm)
Bone age below 12 years (n=5)							
X	12 35	11 80	172 98	2 56	178 58	187 50	8 92
Min	11 32	11 00	170 00	2 00	176 70	184 50	7 40
Max	13 50	12 00	177 00	3 32	181 20	193 00	11 80
S D	0 87	0 45	2 54	0 58	1 80	3 39	1 75
Bone age 12 1–13 0 years (n=11)							
X	12 85	12 85	175 84	1 66	178 95	184 44	5 49
Min	10 32	12 30	169 50	1 00	171 60	181 40	3 50
Max	15 50	13 00	179 40	2 80	183 50	189 00	6 90
S D	1 35	0 27	2 95	0 68	3 73	2 90	2 12
Bone age above 13 years (n=1)							
	12 80	13 5	177 20	2 24	181 00	182 00	1 0

Difference is statistically significant ($p<0.02$)

Table 3 *Results with regard to menarche*

	Chron age (y)	Bone age (y)	Height (cm)	Duration of therapy (y)	Final height (cm)	Growth prognosis (cm)	Growth reduction (cm)
Therapy started before menarche (n=12)							
X	12 69	12 46	175 51	1 85	179 90	186 33	6 43
Min	10 31	11 00	170 00	1 00	176 70	181 40	3 50
Max	15 50	13 00	179 40	3 32	183 50	193 00	11 80
S D	1 39	0 66	3 08	0 76	2 61	3 25	2 55
Therapy started after menarche (n=5)							
X	12 72	12 86	174 04	2 23	176 72	182 48	5 76
Min	13 32	12 30	169 50	1 00	171 60	182 00	1 00
Max	16 16	13 50	177 70	2 80	181 00	184 40	10 40
S D	0 43	0 47	2 91	0 72	3 41	1 07	3 66

1 cm/year Growth after discontinuation of therapy was about 1 2 cm (range 0 4–2 8 cm) The decrease of growth velocity and prognosis is illustrated in the two curves in Fig 2

To date 13 girls have been re examined after discontinuation of therapy All had regular menstrual bleeding within six months In seven girls basal body temperature was reliably measured for some months None had proven ovulatory cycles and some had a cycle with an insufficient corpus luteum period

Side effects of therapy

There were no serious or irreversible side effects of estrogen therapy during the period of observation Almost all the girls had an increase in body weight averaging 11 1 kg but most did not become overweight as they were underweight before therapy After therapy weight loss averaged 6 5 kg Related to chronological age body weight was on the 90th percentile at the beginning and between 90th and 97th percentile after therapy whereas height was above 97th percentile

During the first 2–3 days of therapy most of the girls had nausea and some vomited An increase of pigmentation of areola and nipples of the breast developed in many but normalized after discontinuation of therapy In 5 out of 36 girls galactorrhea was observed for a few days but disappeared spontaneously during unaltered therapy In 3 girls a pre existent small goiter became bigger but decreased in size after substitution with thyroid hormone Acne vulgaris consistently vanished during estrogen therapy but reappeared after discontinuation in some girls In one girl therapy was stopped because of severe headache which immediately disappeared

DISCUSSION

Since the first report by Goldzieher (12) many other studies on estrogen treatment for tall

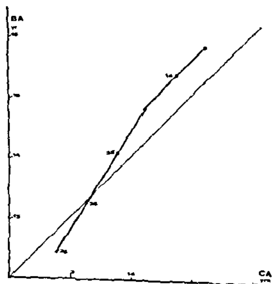


Fig 1 Progress of bone age during estrogen therapy (numbers indicate observations for each point on the figure)

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There was no significant difference in the height finally achieved whether the start of therapy came before or after menarche In the former case 6 43 cm were saved in the latter 5 76 cm (Table 3)

During the first phase of estrogen therapy skeletal maturation advanced more rapidly than the chronological age Later both advanced at the same speed (Fig 1) Growth velocity was reduced to about 3 cm/year during the first six months of therapy to about 2 cm/year during the second six months of therapy and in the following period to about

Table 2 *Results in relation to bone age at start of therapy*

	Chron age (y)	Bone age (y)	Height (cm)	Duration of therapy (y)	Final height (cm)	Growth prognosis (cm)	Growth reduction (cm)
Bone age below 12 years (n=5)							
$\bar{X}$	12 35	11 80	172 98	2 56	178 58	187 50	8 92
Min	11 32	11 00	170 00	2 00	176 70	184 50	7 40
Max	13 50	12 00	177 00	3 32	181 20	193 00	11 80
S D	0 87	0 45	2 54	0 58	1 80	3 39	1 75
Bone age 12 1–13 0 years (n=11)							
$\bar{X}$	12 85	12 85	175 84	1 66	178 95	184 44	5 49
Min	10 32	12 30	169 50	1 00	171 60	181 40	3 50
Max	15 50	13 00	179 40	2 80	183 50	189 00	6 90
S D	1 35	0 27	2 95	0 68	3 73	2 90	2 12
Bone age above 13 years (n=1)							
	12 80	13 5	177 20	2 24	181 00	182 00	1 0

Difference is statistically significant ($p<0\ 02$)

Table 3 *Results with regard to menarche*

	Chron age (y)	Bone age (y)	Height (cm)	Duration of therapy (y)	Final height (cm)	Growth prognosis (cm)	Growth reduction (cm)
Therapy started before menarche (n=12)							
X	12 69	12 46	175 51	1 85	179 90	186 33	6 43
Min	10 31	11 00	170 00	1 00	176 70	181 40	3 50
Max	15 50	13 00	179 40	3 32	183 50	193 00	11 80
S D	1 39	0 66	3 08	0 76	2 61	3 25	2 55
Therapy started after menarche (n=5)							
X	12 72	12 86	174 04	2 23	176 72	187 48	5 76
Min	13 32	12 30	169 50	1 00	171 60	18[illegible] 00	1 00
Max	16 16	13 50	177 20	2 80	181 00	184 40	10 40
S D	0 43	0 47	2 91	0 72	3 41	1 07	3 66

1 cm/year Growth after discontinuation of therapy was about 1 2 cm (range 0 4–2 8 cm) The decrease of growth velocity and prognosis is illustrated in the two curves in Fig 2

To date 13 girls have been re examined after discontinuation of therapy All had regular menstrual bleeding within six months In seven girls basal body temperature was reliably measured for some months None had proven ovulatory cycles and some had a cycle with an insufficient corpus luteum period

Side effects of therapy

There were no serious or irreversible side effects of estrogen therapy during the period of observation Almost all the girls had an increase in body weight averaging 11 1 kg but most did not become overweight as they were underweight before therapy After therapy weight loss averaged 6 5 kg Related to chronological age body weight was on the 90th percentile at the beginning and between 90th and 97th percentile after therapy whereas height was above 97th percentile

During the first 2–3 days of therapy most of the girls had nausea and some vomited An increase of pigmentation of areola and nipples of the breast developed in many but normalized after discontinuation of therapy In 5 out of 36 girls galactorrhea was observed for a few days but disappeared spontaneously during unaltered therapy In 3 girls a pre existent small goiter became bigger but decreased in size after substitution with thyroid hormone *Acne vulgaris* consistently vanished during estrogen therapy but reappeared after discontinuation in some girls In one girl therapy was stopped because of severe headache which immediately disappeared

DISCUSSION

Since the first report by Goldzieher (12) many other studies on estrogen treatment for tall

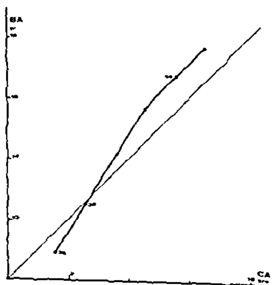


Fig 1 Progress of bone age during estrogen therapy (numbers indicate observations for each point on the figure)

Table 1 *Over all results for patients with completed treatment (n=17)*

	Chron age (y)	Bone age (y)	Height (cm)	Duration of therapy (y)	Final height (cm)	Growth prognosis (cm)	Growth reduction (cm)
X	12 70	12 58	175 08	1 96	178 96	185 19	6 23
Min	10 32	11 00	169 50	1 00	171 60	181 40	1 00
Max	15 50	13 50	179 40	3 32	183 50	193 00	11 80
S D	1 17	0 62	3 02	0 74	3 14	3 29	2 82

tinued until the skeletal age was 15 years or more At first we used mestranol in a few cases but subsequently ethinylestradiol (Lynoral* Progynon M* 0 5 mg/day) for the first 25 days of each cycle and norethisterone (Primolut N* 15 mg/day) for days 21 to 25 were given This was followed by five days without treatment (days 26–30) 13 girls had follow up examinations for 1 5 years after therapy had been stopped

Statistical evaluation was based on the nonparametric Mann Whitney *u* test (7)

RESULTS

Treatment of seventeen girls has been completed At the beginning their mean chronological age was 12 7 years their mean bone age was 12 58 years and their mean height 175 08 cm Their mean predicted height was 185 19 cm After about two years of treatment their mean height was 178 96 cm 6 23 cm less than the predicted height Data (mean range and standards deviation) are presented in Table 1

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Bone age 12 1–13 0 years (n=11)							
X	12 85	12 85	175 84	1 66	178 95	184 44	5 49
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S D	1 35	0 27	2 95	0 68	3 73	2 90	2 12
Bone age above 13 years (n=1)							
	12 80	13 5	177 20	2 24	181 00	182 00	1 0

Difference is statistically significant ($p<0\ 02$)

Table 4 *Comparison of our results with those in the literature*

Author	n	Estrogen	Dosage (mg/day)	Cyclic	Reduction (cm)	Bone age velocity (years/year)
Goldzieher 1956	14	Stilbestrol Conj estrogens	2 0 2 0–10 0			
Freed 1958	2	Conj estrogens	3 75–4 5	No	4 0	2 6
Bayley et al 196	7	Conj estrogens Synth estrogens (stilbestrol ethinylestradiol)	1 25 3 0–5 0 0 15	Yes	1 0	1 ‹6
Greenblatt et al 1966	?4	Conj estrogens	10 0–15 0	No	5 08	
Frasier & Smith 1968	17	Conj estrogens	4 2	Yes	2.5	
Whitelaw 1969	54	Estradiol valerates	3×10 mg/month	Yes	5 0–17 5	2 75
Bierich & Schonberg 1973	11	*Conj estrogens*	7 5	No	5 5	
Schon et al 1973	?1	Conj estrogens	10 0	Yes	4 0	
Job & Joan 1974	?4	Synth estrogens (stilbestrol ethinylestradiol)	 ? 0–10 0 0 0?5–0 1	Differently	4 2	1 7
Wettenhall et al 1975	87	Synth estrogens (stilbestrol ethinylestradiol)	 3 0 0 1?	No	3 5	
Zachmann et al 1975	40	Synth estrogens (ethinylestradiol)	0 3	No	4 6	1 4
Our results 1976	17	Synth estrogens (ethinylestradiol)	0 5	Yes	6 ?3	1 6

of Tanner & Whitehouse (27) Taking this in to account our mean growth reduction of 6 23 cm is well in line with his results

Acceleration of skeletal development during therapy has commonly been found to be between 1 4 and 2 8 years/year (2 18 31 35) In our patients it was 1 6 years/year during the first year of treatment At the same time growth velocity is reduced (18 24 35) presumably due to reduced secretion of somatomedin during estrogen therapy (5 34)

The effect of different estrogen doses on growth are listed in Table 4 Synthetic estrogens in amounts smaller than those used by us also resulted in a significant reduction in final height (35) Observations on girls with scoliosis (20 21) cannot be compared because deformities of the spine interfere with estimation of final height

Serious side effects of estrogen therapy have not been observed so far In particular no sterility due to high estrogen doses interfering with the hypothalamic–pituitary–ovarian axis has not been documented and there are reports of successful pregnancies in women who had previously been treated for excessive height (8 10 28) Like others (13 18 26 28 35) we regularly saw the reappearance of normal menstrual bleeding once therapy had been stopped Since at this age ovulatory cycles are observed in only 10–16% of all girls (9) the finding of anovulatory cycles in our seven patients was not unexpected

We consider estrogen therapy to be justified in patients who are likely to be excessively tall For girls already taller than 160 cm at the age of ten years a first growth prognosis

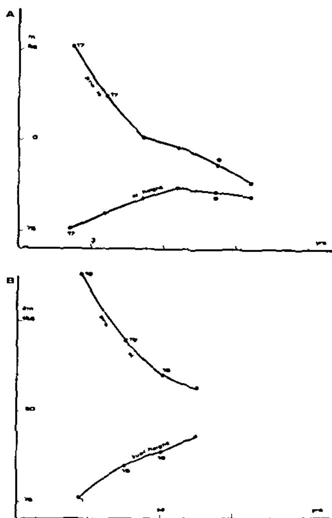


Fig ? Changes of predicted height with longitudinal growth *during estrogen therapy for patients with com* pleted therapy (A) and patients still under treatment (B) The decline in actual height in (A) between 14 and 15 years is due to the decreasing number of patients in the re spective subgroups (numbers indicate observations for each point on the figure)

stature in girls have been published (2 4 5 10 11 12 13 18 22 23 24 26 28 29 30 31 32 33 35) but there are still open questions especially with regard to possible late side ef fects *We therefore only started treatment in* girls with a growth prognosis of 185 cm or more although a lower limit has been chosen in Southern Germany and Switzerland (*5 24 35*) *where average adult height is less than in* Northern Europe (6) For girls with ortho paedic complications growth prognosis was not taken into account since further longi *tudinal growth had not been reduced*

The significance of results depends on the accuracy of height prediction *In accordance* with Zachmann (35) our impression is that future height is slightly overestimated using *the method of Bayley & Pinneau (1) but others (10 26 28) have found close agreement* between estimated and final height A longi tudinal growth study at the Fels Research Institute (Yellow Springs) and the Harvard *School of Public Health (Boston) on girls aged* between 10 and 14 years showed an under estimate of final height when the Bayley and Pinneau method was used but good agree ment was found at the Child Research Council (Denver) (25) The height predictions based on Tanner and Whitehouse II RUS (27) bone age estimations proved to be more accurate than *those calculated* according to Bayley & Pin neau (35) Another point is the accuracy in de termining bone age Since this can be difficult for the period shortly before or during the *adolescent growth spurt we took two or more X ray films of the wrist during the year before* therapy whenever possible to have more than one prediction of final height as a basis for the *decision on whether or not to treat*

Some of the results of estrogen therapy in young girls reported in the literature are listed in Table 4 and compare favourably with our observations There is no certain difference between the effects of cyclic or continuous estrogen treatment of final height We pref erred cyclic therapy to avoid possible hyper trophy of the endometrium *(3) and like others* we added norethisterone Since there is a pos sible connection between vaginal carcinoma in young women whose mothers had been treated with stilbestrol during pregnancy (14 *15 17 19) this estrogen is no longer recom* mended

In general reduction of final height has been more *successful when estrogen therapy has* been started before a skeletal age of 12 5 years (13 18 26 28 35) Starting therapy before or after menarche did not alter our results and this has been observed by others (28 35) Zachmann (35) found a mean growth reduc tion of 4 26 cm using *the prediction method*

Table 4 *Comparison of our results with those in the literature*

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We consider estrogen therapy to be justified in patients who are likely to be excessively tall For girls already taller than 160 cm at the age of ten years a first growth prognosis

should be established If repeated predictions confirm the probability of excessive growth estrogen therapy can be started at a bone age between 11 and 12 years in time for a good chance of successful reduction in final height

ACKNOWLEDGEMENT

The authors wish to thank Prof A Lassrich and Dr E Richter for evaluation of X ray films

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Submitted May 25 1976
Accepted Sept 14 1976

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Acta Pædiatr Scand 66 169–175 1977

LEAD IN UMBILICAL CORD BLOOD CORRELATED WITH THE BLOOD LEAD OF THE MOTHER IN AREAS WITH LOW MEDIUM OR HIGH ATMOSPHERIC POLLUTION

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ABSTRACT Zetterlund B Winberg J Lundgren G & Johansson C (Departments of Paediatrics and Analytical Chemistry University of Umeå Sweden) Lead in umbilical cord blood correlated with the blood lead of the mother in areas with low medium or high atmospheric pollution Acta Paediatr Scand 66 169 1977.— Lead concentrations in 541 samples of umbilical cord blood from different parts of Sweden were determined The mean concentration was 7 6 μg lead/100 ml (=0 367 μmol/l) The blood lead values were also determined for 297 mothers and a mean value of 8 7 μg/100 ml (=0 420 μmol/l) was found There was a significant correlation between the blood lead level of the mother and the infant as studied in 253 pairs The slope of the regression line was 0 5 r (r=0 6) Significantly lower blood values for both mother and infant were found in areas with low pollution as estimated from the lead content in moss No seasonal variation could be ascertained Hematocrit versus lead concentration was also studied A flameless atomic absorption method was used with a standard deviation of 0 9 μg lead/100 ml The storage time and sample treatment were also studied

KEY WORDS Lead newborn infants maternal–fetal exchange environmental pollutants

The environmental health risks from exposure to metals or metal compounds have been a matter for serious concern during recent years (31) The childhood exposure to environmental lead has been studied by Darrow et al (8) who also reviewed other works Sayre et al (26) studied dust as a source for childhood exposure There has however been little attention given to the exposure of fetuses or infants and very few data are available as pointed out by Scanlon (27) The metal intake in early life should therefore be investigated as it is expected that the effect of metals is larger on rapidly growing tissue than on that of the adult individual (5 18) Lead intoxication in utero is wellknown and there are many reports in the literature (32) Other studies have indicated a placental transfer of lead in absence of obvious intoxication symptoms of mother or infant (11 12 15 17 23 24 28)

Significant differences between urban and suburban infants or infants with smoking and nonsmoking mothers have not been demonstrated in these studies

The number of patients studied have often been small and the accuracy and sensitivity of the analytical methods used have not always been appropriate since they have been developed mainly for patients with professional lead exposure The blood levels to be expected in newborns are much lower than those found in professionally exposed workers The method to be selected should therefore have a detection limit of at least 1 μg/100 ml blood Several methods have been used for the determination of lead in blood e g spectrographic

Table 3 *Effect of storage time on the blood lead value (µg lead/100 ml)*

R2–R8 are professionally exposed and are not included in our material 1 µg/100 ml=0 048 µmol/l

Sample	Initial value	Number of months after first analysis 1	2	3	5	6
R2	47	40	45			
R3	45	43	43			
R4	46	43	43			
R6	24	20		23		
R7	18	19	23			
R8	34	30		32		
R12	7	5	6	6	7	6
R13	7				6	7
R14	5				6	5
R15	5				6	5

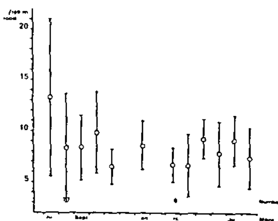


Fig 2 Seasonal variations of lead concentrations in cord blood Umeå 1973–1974 Mean values±1 S D 336 samples 1 µg/100 ml=0 048 µmol/l

Analytical methods

Lead was analyzed by flameless atomic absorption spectroscopy using instrumentation which has been described earlier (19 20) as well as on a Varian AA6 with a CRA 63 graphite furnace On analysis with our own graphite furnace the blood was diluted to ten times its original volume with water using a pneumatic dilutor To 1 0 ml of diluted blood 50 µl of 2% Triton X 100 was added to ensure total haemolysis of the red blood cells A small aliquot (1–5 µl) of the sample was placed in the graphite tube atomizer with a micropipette (Unimetric or Oxford sampler) The working parameters for a lead determination were drying for 30 sec at 100°C ashing for 30 sec at 650°C and atomization at 1 300°C

The procedure used with the CRA 63 was identical except that the sample was diluted to three times its original volume with 2% Triton X 100 The difference in procedure is due to different sensitivity and differences in the formation of particles with non specific absorption

A calibration curve was obtained from standard additions of small volumes of lead nitrate stock solution The reproducibility at low levels is shown in Table 2 the standard deviation of the analytical method was 0 9 µg lead/100 ml 22 blood samples from workers at a lead melting plant (Boliden AB Sweden) were analyzed and compared with the results obtained by their analytical department using an extraction method (34) The largest difference was 2 µg/100 ml at a level of 10–48 µg/100 ml (1 µg/100 ml=0 048 µmol/l) The delay before analysis should be of little importance for the result as shown by Table 3 If the samples were partially coagulated the results were different even on the same day and in order to obtain reliable values such samples must be discarded without hesitation Attempts to dissolve such samples in acids were not successful

Hematocrit was estimated by centrifugation of microtubes at 11 000 RPM for 4–5 min

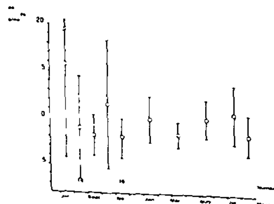


Fig 1 Seasonal variations of lead concentrations in maternal blood Umeå 1973–1974 Mean values±1 S D 1?7 samples 1 µg/100 ml=0 048 µmol/l

RESULTS

In four maternal blood samples out of 297 (1 3%) and in eight out of 541 cord blood specimens (1 5%) the lead concentration was above 20 µg/100 ml blood The mean concentration was 8 7 µg/100 ml (=0 420 µmol/l) for the mothers and 7 6 µg/100 (=0 367 µmol/l) for the fetuses Seasonal variations of maternal and fetal lead levels in the samples from Umeå are shown in Figs 1 and 2 No definite conclusions can be drawn from the results The high mean levels found in July 1973 for both mothers and infants are based on eight samples Since these first eight determinations belonged to the 15 highest values found in the

Table 1 *Source of samples and lead content of the moss in the area*

Geographical group		No of specimens Cord	Maternal	Index of atmospheric pollution (µg lead/kg moss)
A	Kiruna	33	14	5–10
	Gallivare	11	6	10
	Lycksele	3	1	20–40
B	Umeå	336	127	40–60
	Skellefteå	18	9	40–60
	Stockholm	37	37	60
C	Helsingborg	32	30	80–100
	Angelholm	13	9	60–80
	Halmstad	8	9	60–80
	Molndal	20	21	60–80
	Uddevalla	30	34	60–80

(7) atomic absorption spectrometry after extraction with APCD in MIBK (14 34) and spectrophotometry using dithizone An adequate sample treatment has been one of the main difficulties Treatment with heparin and triton has been applied but still the analysis presents several pitfalls as illustrated by a recent review (2) as well as a comparison between different techniques (1) It has been shown that flameless atomic absorption in a graphite tube can provide reliable results provided a background correction is used in a two channel instrument (19–21) The background correction subtracts non selective absorption due to smoke or particles The temperature must be controlled during the ashing step in order to perform the ashing at a temperature just below that at which losses occur In this way the non specific absorption is reduced and accuracy is improved Difficulties with the formation of ash on the graphite surface still remain (10)

The present study was planned so that samples would be obtained from areas with various atmospheric lead levels including urban as well as suburban regions The relation between maternal and fetus blood levels was also studied Sources other than atmospheric lead pollution may be more important but data for comparisons were not available The present material was not examined for clinical symptoms since no such effects were expected at the lead levels found The results are presented in µg/100 ml blood 1 µg/100 ml= 0 048 µmol/l

MATERIAL

Blood samples were collected in Umeå from July 1973 until August 1974 and in other places in Sweden from October 1973 until November 1974 541 cord blood samples and 297 samples from delivered mothers were analyzed Blood samples were obtained from the maternity clinics in the cities listed in Table 1 The patients (age 16–43 years mean 25 6 years) lived in the corresponding cities or in the surrounding region These places were chosen because they represented regions with low intermediate and high atmospheric pollution of lead according to Tyler & Ruhling (25) cf Table 1 The pollution was estimated from the lead content of a moss (*Hylocomium splendens*) which reflects the pollution during the 5–6 years preceding collection (30) The moss was collected (1968–1969) at places situated at least 300 m from a road Lead from motor car fuel was judged not to have influenced the values

METHODS

Blood sampling

Maternal venous blood was collected just after delivery into heparinized test tubes (Vacutainer L 320 XF 313) Cord blood was allowed to flow directly into the test tube The specimens were kept at +4 C until analysis was performed During transportation temperature was not checked The most critical operation was found to be the shaking of the Vacutainer tubes just after the sampling If not properly performed the blood would coagulate during transport

Test tubes stoppers and canulas did not yield measurable quantities of lead when washed in HNO_3 for 24 hours The heparin was free from lead

Table 2 *Variation in samples with low levels of lead (cord blood)*

1 µg/100 ml=0 048 µmol/l

Sample number	Lead concentration (µg/100 ml)				Mean
961	5 3	5 6	5 6	5 3	5 5
1116	4 4	3 7	4 4	4 0	4 1
1118	4 0	4 0	3 7	4 4	4 0
1119	5 1	4 8	4 0	4 8	4 7
1131	4 2	5 6	5 3	4 9	5 0

sis separately for each geographical region No variation was found The possibility that the quotient may differ at different maternal lead levels was also tested and rejected The age of the mother did not influence the lead level of her blood or that of her infant

As it is supposed (6 24) that more than 95% of the lead in the blood is bound to the red cells the values of the infants were correlated with the hematocrit see Fig 4 A correlation coefficient of 0 19 was obtained and therefore the variations seen in cord blood/maternal blood quotient cannot be explained in this way In an examination of blood from 16 mothers no correlation was found between lead value and hematocrit (r=0 08) These results are supported by Kochen & Greener (15) who stated that hematocrit is of little importance for lead uptake in blood when lead levels are below 250 µg/100 ml (=12 µmol/l)

DISCUSSION

The mechanism for transfer of lead from mother to infant is unknown In two studies (11 12) a slightly higher concentration has been found in maternal blood than in fetal cord blood No conclusions about the transport mechanism can be drawn from these data or from the autopsy material published by Schroeder & Tipton (29)

Studies on rat fetuses after a single injection

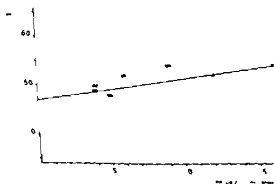


Fig 4 Hematocrit and lead concentration in 57 cord blood samples Correlation coefficient=0 19 1µg/100 ml =0 048 µmol/l

Table 5 *Reported values for lead in blood in new born infants (µg/100 ml)*

1 µg/100 ml=0 048 µmol/l

Study	n	Mean	Range
Harris & Holley	24	12 3	10–20
Scarlon	28	20 1	10–39
Kubasik et al	20	13 8	7–23
Rajegowda et al	100	14 6	10–30
Kochen et al	35	19 1	12–47
Haas et al	294	14 98	1–32
This report	541	7 6	2–25

Estimated from the figure

of lead 203 (16) cannot imitate the long low dose exposure of the ordinary pregnant woman

This study has shown that in the majority of 297 maternal and 541 cord blood samples the lead concentration was between 5 and 15 µg/100 ml blood (0 241–0 724 µmol/l) The values were lower in areas with low atmospheric pollution There are few reports in the literature on cord blood lead levels Table 5 summarizes some published data The present Swedish data especially those from the northern part of the country seem to be much lower than the American and German ones If the difference is real or due to methodological discrepancies is uncertain

Estimates of the natural lead concentration in organic materials have suggested a natural human blood concentration of 0 025 µg/100 ml (0 001 µmol/l) (22) Compared with this the present values are high Biochemical effects of low lead concentrations have been demonstrated by Hernberg & Nikkanen (13) who found a decreased activity of deltaaminolevulinic acid dehydratase in erythrocytes at blood lead values between 5 and 15 µg/100 ml

A crucial question is however whether the blood lead levels found in the present and in other studies may be clinically harmful in the long run Little is known about risks of long term exposure to low levels this applies especially to fetuses and infants Encephalopathy in children is seen after acute lead intoxication but a few studies suggesting cerebral symp

Table 4 *Concentration of lead (±1 S D) in maternal and cord blood in relation to the used index of atmospheric pollution*

1 μg/100 ml=0 048 μmol/l

Area	Atmospheric pollution	Maternal blood samples No	Maternal blood samples Mean conc μg Pb/100 ml	Cord blood samples No	Cord blood samples Mean conc μg Pb/100 ml
A	Low	21	6 1±2 1	47	4 4±2 0
B	Intermediate	173	9 2±3 7	391	8 0±3 8
C	High	103	8 4±2 7	103	7 3±2 7

37 samples from a big city (Stockholm) gave a mean of 9 4 μg Pb/100 ml blood for the mothers and 6 8 μg Pb/100 ml for the cord blood

whole study (838) samples) an error cannot be excluded

Geographical variations in lead levels were then analyzed Table 4 compares the lead content of maternal and of cord blood for geographical regions with different grades of atmospheric pollutions The lowest mean value was obtained from the northern part of the country (area A) where pollution is low The difference was highly significant ($p<0\ 001$) for both mothers and infants in comparison with regions with intermediate (B) and high (C) pollution In the latter areas maternal levels did not differ significantly Unexpectedly the blood lead concentrations were lower in the highly polluted region C than in region B ($p<0\ 05$ for cord blood) To see whether this could be explained by the fact that part of the population in area B lived in a big city (Stockholm) the mean values for these individuals were calculated separately see Table 4

The lead concentration of the maternal blood was then plotted versus that of the fetal blood for 253 mother and infant pairs see Fig 3 The slope of the regression line was 0 53 ($r=0\ 62$) the fetal level being lower than the maternal one A large number of values fall far from the regression line This indicates that factors other than the actual blood concentration of the mother influence the cord blood level The correlation between fetal and maternal blood was submitted to statistical analy

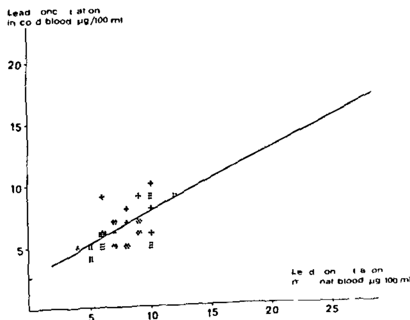


Fig 3 Correlation between maternal and fetal blood lead concentrations for 253 mother–infant pairs Correlation coefficient =0 62 1 μg/100 ml=0 048 μmol/l

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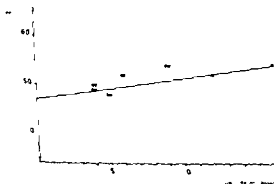


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1 µg/100 ml=0 048 µmol/l

Study	n	Mean	Range
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Scanlon	8	?0 1	10–39
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toms after sublinical lead exposures are also published David et al (9) reported slightly higher blood lead values in a group of hyper active children compared with controls but there is no proof of a causal relation between the hyperactivity and lead Another report (3) suggests correlation between mental retardation and lead content in drinking water in Glas gow In both of these investigations the con trols had blood lead levels of 17 and 23 μg/100 ml and the affected children 24 and 26 μg/100 ml In a follow up study de la Burde et al (4) found impaired cognitive perceptual and behavioural functioning in a group of children 4–6 years after proved lead exposure without acute symptoms The observed group had blood lead levels exceeding 40 μg/100 ml at the exposure

Although the levels reported here are lower than those reported from several other coun tries there is still reason for concern about the increasing lead exposure (33) especially for fetuses and small children It thus seems war ranted to follow the impact of this regularly for example by (*a*) surveying lead concentra tions and sources of lead exposure (*b*) to look for possible clinical effects especially long term consequences and (*c*) to learn more about transport and biochemical effects of lead and lead compounds in the body

ACKNOWLEDGEMENTS

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Submitted June 3 1976
Accepted Sept 21 1976

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Acta Pædiatr Scand 66 177–184 1977

C PEPTIDE IN JUVENILE DIABETICS BEYOND THE POSTINITIAL REMISSION PERIOD

Relation to Clinical Manifestations at Onset of Diabetes Remission and Diabetic Control

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From the Department of Paediatrics Linkoping University Sweden [1]Novo Research Institute Copenhagen Denmark and the [3]Department of Mathematics Linkoping University Sweden

ABSTRACT Ludvigsson J Heding L G Larsson Y and Leander E (Department of Paediatrics Linkoping University Sweden Novo Research Institute Copenhagen Denmark Department of Mathematics Linkoping University Sweden) C peptide in juvenile diabetics beyond the postinitial remission period —Relation to clinical manifestations at onset of diabetes remission and diabetic control Acta Paediatr Scand 66 177 1977 —A group of 58 diabetics age 6–17 years and with a duration of diabetes of 3–14 years was studied in order to show whether the nature of the clinical manifestations and the treatment at the onset of the disease are related to the subsequent C peptide production and also whether remaining C peptide production is related to better diabetic control The relations between a number of clinical and laboratory variables were analysed including the degree of ketosis and the insulin dose given at onset of diabetes the incidence of postinitial remission period the fasting C peptide level after the remission period the level of insulin antibodies and the actual diabetic control expressed as the degree of glucosuria in the patients urine tests at home Multiple regression analysis was the main method used Postinitial remission was positively correlated to initial insulin dose and negatively correlated to duration of ketonuria at onset C peptide which was found in 24 1% of the patients was positively correlated to age at onset and initial insulin dose but negatively correlated to ketonuria at onset Diabetic control was positively correlated to insulin dose at onset and to C peptide level but negatively correlated to insulin antibodies It could further be shown that patients who had received a more vigorous treatment immediately at onset had both a higher incidence of postinitial remission and a better diabetic control The results suggest that an early diagnosis followed by rapid normalization of the metabolism at the onset of juvenile diabetes increase the possibility of preservation of some of the endogenous insulin production which seems to facilitate diabetic control

KEY WORDS Juvenile diabetes ketonuria insulin remission C peptide insulin antibodies

C peptide is considered to reflect the insulin production of the beta cells (2 14 16 22) In juvenile insulin requiring diabetics C peptide levels equal to those present in non diabetics have been demonstrated in the postinitial remission period (1 3 9 13) Thereafter such patients are considered to have more or less completely lost their capacity for endogenous insulin secretion (7 21) However we were recently able to show that in a group of 96 diabetic children with more than two years of duration measurable amounts of C peptide were present in 35 4% of the patients (19) This indicates that a residual beta cell function may exist even for prolonged periods in some cases of juvenile diabetes It seemed to be of interest to study in more detail such cases and particularly to analyse whether the nature of

Table 3 *Duration of ketonuria at onset of diabetes*

Onset	Days 0	1–3	4–6	7–9	>9	Mean days	S D	Total *no*
Before Jan 1st 1970	4	8	8	7	12	6 9	5.5	39
After Jan 1st 1970	4	9	5		1	2 9	3 3	19
Total	8	17	13	7	13	5 7	5 2	58
%	13 8	29 3	22 4	12 1	22 4			100 0

METHODS

Clinical data were taken from patient records supplemented by questionnaires regarding heredity The degree of diabetic control was determined on the basis of the urinalysis made at home From the patients diaries during 1975 the number of tests showing less than 1% glucosuria was counted and expressed in percentage of all tests performed This figure was taken as an index of diabetic control Consequently a high index figure indicates a more normal glucose metabolism than a low figure

Postinitial remission was defined as the period following the first weeks of hospital treatment in which more than 95% of the urine tests at home and at the hospital showed less than 1% glucosuria for more than one week During this period the insulin requirements were low with a mean of 0 5 and a range of 0 0–0 9 IU/kg body weight For comparison the corresponding figures for 1975 were mean 1 1 and range 0 6–1 8 IU/kg body weight

Laboratory methods Blood was drawn from the fasting patients prior to their morning insulin injection Serum used for determination of C peptide IgG and IRI was stored at −18 C

C peptide was determined by a radioimmunoassay in which proinsulin is being removed through binding to insulin antibodies covalently coupled to Sepharose (12) Since most of the serum contained insulin antibodies which have been shown to bind proinsulin the serum was extracted at a low pH in the same way as described for total IRI (11) before the determination of C peptide Detection limit is 0 03 *pmol/ml* All serum samples were assayed in triplicates using antisera M1181 (12)

The insulin binding capacity of IgG was determined by the method of Christiansen (5) and total immunoreactive insulin IRI was determined after acid extraction of serum according to Heding (11)

HLA typing was performed by the lymphocytotoxic microtechnique described by Kissmeyer Nielsen and Kjerbye (15) Lymphocytes were isolated according to Thorsby (24) and Bøyum (4)

Statistical methods Multiple regression analysis was the main technique employed The following variables were included in the evaluation family history of juvenile diabetes mellitus family history of maturity onset diabetes mellitus HLA type (B8 and/or BW 15) sex age at onset of diabetes (age at onset of diabetes)² onset of diabetes before January 1st 1970 duration of diabetes (duration of diabetes)² infection before onset of diabetes blood glucose levels on first admission to hospital occurrence of severe ketoacidosis on first admission to hospital ketonuria on first admission to hospital duration of ketonuria at onset insulin dose during the first 24 hours at hospital (IU/kg body weight) occurrence of remission period square root of fasting serum C peptide index of diabetic control (during 1975) insulin binding capacity of IgG and total serum insulin IRI

RESULTS

Manifestations at onset Infections had according to available information preceded the onset of diabetes by at most two months in 16 patients (27 6%) In 4 asymptomatic patients (6 9%) the disease was diagnosed by chance at routine medical examination while the remaining 54 patients (93 1%) had had diabetic symptoms for an average of 20 days (range 2–120) before admission to hospital Nine patients (15 5%) were admitted to hospital with severe

Table 4 *Duration of the postinitial remission period*

	Days 0	8–30	31–60	61–120	>120	Mean	Range	Total no
Boys	18	6	1	4	3	62	9–190	32
Girls	22	4	0	0	0	20	18–21	6
Total	40	10	1	4	3	53	9–190	58
%	69 0	17 2	1 7	6 9	5 2			100 0

Table 1 *Heredity for diabetes (DM)*

	Diabetes among					
	Parents		Siblings		Grandparents	
	n	%	n	%	n	%
Juvenile DM	4	6 9	6	10 3	0	
Maturity onset DM	1	1 7	0		17	29 3
Juvenile+maturity onset DM	0		0		0	
No DM	*53*	*91 4*	*52*	*89 7*	*41*	*70 7*
	58	100 0	58	100 0	58	100 0

the clinical manifestations and the treatment at the onset of the disease are related to the subsequent C peptide production and also whether remaining C peptide production is related to better diabetic control

MATERIAL

A sample of 58 insulin treated juvenile diabetics 6–17 years of age was studied All cases had passed the post initial remission period and the duration of diabetes varied from 3–14 years (mean 7 3±3 0) Age at onset of diabetes varied between 1–13 years (mean 6 0±3 4) Family history for diabetes is shown in Table 1 All patients were regularly seen at the diabetic clinic of the pediatric department 4–8 times a year In the statistical evaluation the patients were divided into two groups 39 patients with onset of diabetes before January 1st 1970 and 19 patients with onset thereafter This division of the material was done because in 1970 there was a change in the therapeutic policy both with regard to the initial treatment at onset and the following diabetic care Before 1970 diabetic patients at onset were given rather small gradually increasing doses of insulin correlated to laboratory data and severity of symptoms of the patient on admission to hospital (Table 2) Relatively prolonged periods of ketonuria were rather common (Table 3) Ketonuria was considered to be present until the urine was continuously negative for two days The aims of the subsequent treatment may be briefly described as clinical well being freedom from symptoms of diabetes and normal growth and development while less attention was paid to the metabolic control Urinalysis at home was not recommended and the diet was only roughly regulated After 1970 the treatment became more intense At onset higher insulin doses were given (Table 2) aiming at a normalization of blood glucose and elimination of ketonuria within 24–28 hours Thereafter the aim was a metabolic state as normal as possible This was achieved through a treatment consisting of insulin regular physical activity regulated diet (with the help of a dietitian) and daily glucosuria control at home Thus after 1970 all patients were instructed to test their urine three times a day usually before breakfast before dinner and in the evening by the Clinitest 2-drops method and to record the results in a diary shown to the doctor at each hospital visit They were also instructed to check their urine for ketone bodies with Ketostix in acute situations By the time of this study 28 patients (48 3%) tested their urine 2–3 times a day 29 patients (50 0%) 1–2 times a day and only one of the 58 patients (1 7%) did not test the urine regularly During both periods the patients were as a rule given one morning dose of intermediate insulin (NPH or Lente) often supplemented by shortacting insulin (Semilente® Actrapid® or Regular insulin) during the first years of diabetes but when this failed to keep them well regulated (usually coinciding with the first signs of puberty) two daily doses were given At the time for C peptide determinations 35 patients (60 3%) had two doses a day Growth and development (somatic psychological and intellectual) was within normal limits in all cases except in one boy with delayed onset of puberty

Table 2 *Insulin treatment during the first 24 hours after admission to hospital at onset of diabetes*

Onset	Insulin dose (IU/kg b wt) ≤0 5	0 6–1 0	1 1–1 5	1 6–2 0	>2 0	Mean (IU/kg)	S D	Total no
Before Jan 1st 1970	16	11	5	4	3	1 0	1 1	39
After Jan 1st 1970	4	6	0	4	5	1 5	1 3	19
Total	20	17	5	8	8	1 1	1 2	58
%	34 5	29 3	8 6	13 8	13 8			100 0

Table 3 *Duration of ketonuria at onset of diabetes*

Onset	Days 0	1–3	4–6	7–9	>9	Mean days	S D	Total no
Before Jan 1st 1970	4	8	8	7	12	6 9	5.5	39
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Total	8	17	13	7	13	5 7	5 2	58
%	13 8	29 3	22 4	12 1	22 4			100 0

METHODS

Clinical data were taken from patient records supplemented by questionnaires regarding heredity The degree of diabetic control was determined on the basis of the urinalysis made at home From the patients diaries during 1975 the number of tests showing less than 1% glucosuria was counted and expressed in percentage of all tests performed This figure was taken as an index of diabetic control Consequently a high index figure indicates a more normal glucose metabolism than a low figure

Postinitial remission was defined as the period following the first weeks of hospital treatment in which more than 95% of the urine tests at home and at the hospital showed less than 1% glucosuria for more than one week During this period the insulin requirements were low with a mean of 0 5 and a range of 0 0–0 9 IU/kg body weight For comparison the corresponding figures for 1975 were mean 1 1 and range 0 6–1 8 IU/kg body weight

Laboratory methods Blood was drawn from the fasting patients prior to their morning insulin injection Serum used for determination of C peptide IgG and IRI was stored at −18 C

C peptide was determined by a radioimmunoassay in which proinsulin is being removed through binding to insulin antibodies covalently coupled to Sepharose (1?) Since most of the serum contained insulin antibodies which have been shown to bind proinsulin the serum was extracted at a low pH in the same way as described for total IRI (11) before the determination of C peptide Detection limit is 0 03 pmol/ml All serum samples were assayed in triplicates using antisera M1181 (1?)

The insulin binding capacity of IgG was determined by the method of Christiansen (5) and total immunoreactive insulin IRI was determined after acid extraction of serum according to Heding (11)

HLA typing was performed by the lymphocytotoxic microtechnique described by Kissmeyer Nielsen and Kjerbye (15) Lymphocytes were isolated according to Thorsby (?4) and Boyum (4)

Statistical methods Multiple regression analysis was the main technique employed The following variables were included in the evaluation family history of juvenile diabetes mellitus family history of maturity onset diabetes mellitus HLA type (B8 and/or BW15) sex age at onset of diabetes (age at onset of diabetes) onset of diabetes before January 1st 1970 duration of diabetes (duration of diabetes) infection before onset of diabetes blood glucose levels on first admission to hospital occurrence of severe ketoacidosis on first admission to hospital ketonuria on first admission to hospital duration of ketonuria at onset insulin dose during the first 24 hours at hospital (IU/kg body weight) occurrence of remission period square root of fasting serum C peptide index of diabetic control (during 1975) insulin binding capacity of IgG and total serum insulin IRI

RESULTS

Manifestations at onset Infections had according to available information preceded the onset of diabetes by at most two months in 16 patients (27 6%) In 4 asymptomatic patients (6 9%) the disease was diagnosed by chance at routine medical examination while the remaining 54 patients (93 1%) had had diabetic symptoms for an average of 20 days (range 2–120) before admission to hospital Nine patients (15 5%) were admitted to hospital with severe

Table 4 *Duration of the postinitial remission period*

	Days 0	8–30	31–60	61–120	>120	Mean	Range	Total no
Boys	18	6	1	4	3	[illegible]	9–190	[illegible]
Girls	[illegible]	4	0	0	0	[illegible]	[illegible]	26
Total	40	10	1	4	3	53	9–190	58
%	69 0	17 [illegible]	1 7	6 9	5 [illegible]			100 0

Table 5 *Diabetic control*

Index=incidence of urine tests with less than 1% glucose

Index	*n*	%
>70%	25	43 9
50–70%	24	42 1
<50%	8	14 0
	57	100 0

symptoms of ketoacidosis including drowsiness but nobody was unconscious On admission ketonuria was present in 50 patients (86 2%) (Table 3) Blood glucose levels on admission to hospital varied between 4 2–66 7 mmol/l (mean 23 5) Insulin doses given during the first 24 hours at hospital are shown in Table 2

Postinitial remission according to the definition used was more common and longer in boys than in girls (Table 4) In 7 patients the remission period exceeded two months

Diabetic control expressed as the glucosuria level in home testing during 1975 could be assessed in the 57 patients who tested their urine regularly In the majority of cases more than 50% of the test showed less than 1% glucosuria (Table 5)

C peptide was measurable (≥0 04 pmol/ml) in 14 patients (24 1%) In comparison with non diabetic children comparable with regard to age weight and sex (18) 5 had values within and 9 below the normal range (0 22–0 73 pmol/ml) In the remaining 44 patients (75 9%) no C peptide could be demonstrated

Insulin antibodies The mean value for IgG was 3 359±2 758 mU/ml (range 0 130–11 029) and for IRI 1 329±1 824 μU/ml (range 10–7 808) The correlation between the two methods was good (r=0 72 p<0 001)

HLA HLA B8 was present in 17 patients (29 3%) and BW15 in 18 patients (31 0%) The combination HLA B8W15 was seen in 4 patients (6 9%) Further details regarding HLA types in this material will be published elsewhere (20)

Partial correlations In the multiple regression analysis special attention was paid to the interdependence of the variables in patients with onset before 1970 and these results are given in detail in Tables 6–9 The tables do not include statistically significant correlations only but also others of importance in the regression analysis From the analysis of patients with onset after January 1st 1970 only statistically significant results are reported as non significant results in this smaller group of patients neither contradict nor confirm results already presented regarding the larger group of patients with onset before 1970

Remission Before 1970 remission periods occurred significantly more often in those patients who had received a relatively higher insulin dose during the first 24 hours at hospital at onset (p>0 02) (Table 6) When patients with onset after 1970 were added this relationship became even more pronounced (r=0 49 p<0 001) and a prolonged period of ketonuria at onset was significantly correlated to an absence of remission period (r=0 39 p<0 005) In addition with all other analysed variables kept constant patients with onset after 1970 had a higher incidence of remission periods (r=0 33 p<0 02)

C peptide The relations between C peptide and other variables are shown in Table 7 The level of C peptide was higher in patients with late onset of diabetes (p<0 001) but the cor

Table 6 *Statistical analysis of the incidence of remission periods in relation to clinical data at onset in 39 patients with onset before January 1 1970*

	t	*r*	*p*
Age at onset	−0 74	−0 14	n s
(Age at onset)²	1 14	0 21	n s
Girls/boys	−1 94	−0 34	<0 10
Infection before onset	*−1 07*	*−0 19*	n s
Occurrence of severe ketoacidotic symptoms	−1 27	−0 23	n s
Incidence of ketonuria	−0 65	−0 12	n s
Blood glucose on admission	−0 22	−0 04	n s
Insulin dose (IU/kg b wt) during the first 24 hours	2 60	0 43	<0 02

Table 7 *Statistical analysis of C peptide in relation to clinical data in patients with onset before 1970*

	All patients n=39			Patients without remission n=32		
	t	r	p	t	r	p
Family history of maturity onset diabetes	−1 47	−0 27	n s	−1 90	−0 38	<0 10
Age at onset	4 23	0 63	<0 001	4 85	0 72	<0 001
Duration of diabetes	1 91	0 35	<0 10	−2 05	0 40	<0 05
(Duration of diabetes)²	−1 99	−0 36	<0 10	−2 09	0 41	<0 05
Infection before onset	−1 68	−0 31	n s	−1 80	−0 36	<0 10
Occurrence of severe ketoacidotic symptoms	−1 94	−0 35	<0 10	−1 57	−0 3?	n s
Blood glucose on admission	−1 81	−0 33	<0 10	−2 09	−0 41	<0 05
Duration of ketonuria	−2 1?	−0 38	<0 05	−2 55	−0 54	<0 0?
Insulin dose (IU/kg b wt) during the first 4 hours	2 87	0 48	<0 01	3 76	0 63	<0 001
Occurrence of remission	−2 71	−0 46	<0 02	–	–	–

relation to duration of diabetes was weak Prolonged ketonuria at onset was related to low levels of C peptide ($p<0\ 05$) while high insulin doses during the first 24 hours at onset were significantly related to high levels of C peptide ($p<0\ 01$) In the patients with onset before 1970 the occurrence of remission periods was negatively correlated to later C peptide level ($p<0\ 02$) but this relation was not found in patients with onset after 1970 Otherwise there were no significant differences in the pattern of relationships between patients with onset before and those with onset after 1970

Diabetic control The relation between the index of diabetic control in 1975 and some of the clinical and laboratory variables are shown in Table 8 Significant correlations were found with regard to duration insulin dose at onset and insulin antibodies When patients with onset after 1970 were added a high index was positively correlated to the amount of C peptide ($r=0\ 30$ $p<0\ 05$) and patients with onset after 1970 had a significantly higher index figure ($r=0\ 39$ $p<0\ 01$)

A summary of the statistical relations is shown in Table 9 Other analysed variables such as family history of diabetes HLA type or infection before onset were not significantly correlated to either remission period C peptide or to diabetic control

DISCUSSION

Methods Multiple regression analysis has been the main technique employed In this type of analysis an effort is made to express values obtained in the dependent variable by a function of background variables Results of statistical analysis may be presented in terms of partial regression coefficients partial correlation coefficients observed values of Student s t and the associated p values For example in the case of the relation between C peptide and insulin dose at onset other variables being held constant the regression coefficient may be considered an estimate of the difference in C peptide for two individuals who differ one unit in insulin dose but are otherwise alike as concerns the background

Table 8 *Statistical analysis of the diabetic control index during 1975 in relation to some variables in 38 patients with onset before January 1 1970*

	t	r	p
Age at onset	−1 96	−0 34	<0 10
Duration of diabetes	? 76	0 44	<0 01
(Duration of diabetes)²	−? 83	−0 45	<0 01
Insulin dose (IU/kg b wt) during the first ?4 hours at onset	? 73	0 44	<0 02
Insulin antibodies (IgG) 1975	−2 89	−0 46	<0 01

Table 9 *Summary of results of multiple regression analysis*

Variables	Type of correlation	*p* values: Onset before 1970	*p* values: All patients
Remission			
Insulin at onset	+	<0.02	<0.001
Ketonuria	–	n.s.	<0.005
Onset after 1970	+	–	<0.02
C peptide			
Age at onset	+	<0.001	<0.001
Insulin at onset	+	<0.01	–
Ketonuria	–	<0.05	–[a]
Remission	–	<0.02	*n.s.*
Control			
Insulin at onset	+	<0.02	–
Onset after 1970	+	–	<0.01
Duration	–	<0.01	<0.01
C peptide	+		<0.05
Insulin antibodies	–	<0.01	<0.01

[a] Not analysed due to change in therapeutic policy

variables under consideration. The correlation coefficient serves as an estimate of the correlation between C peptide and insulin dose in samples comprising individuals who are alike with respect to the remaining background variables. The *t* value is obtained when testing the null hypothesis stating that any correlation between C peptide and insulin dose is of a random nature when other background variables are held constant. The *p* values yield information on the frequency of a partial correlation (or *t* value) of the size observed supposing the relationship is purely random.

In this investigation we have used the square root of C peptide as a dependent variable rather than C peptide. Such a transformation is chosen to counteract the tendency of results being dominated by a few individuals with much higher C peptide values than the rest. In consequence, however, partial regression coefficients are not very informative as the scale of measurement is transformed. Instead of such coefficients partial correlation coefficients which are less closely associated to scales of measurement have been presented. The variables (age at onset)^2 and (duration of diabetes)^2 have been included to make it possible to express non-linear relationships between these and other variables. In a retrospective study it is difficult to get reliable information about anamnestic data such as preceding infections, which to some extent may explain lack of correlations to other variables.

Results. During the remission period the beta cells produce C peptide and insulin in equimolar amounts (1, 3, 9, 13). However, even beyond the termination of this period measurable amounts of C peptide were found in 14 out of 58 patients (24.1%). Patients with prolonged ketonuria at onset and/or low insulin dose during the first 24 hours at hospital did seldom develop significant remission periods and had lower levels of C peptide later on. Patients with severe ketoacidotic symptoms and high blood glucose levels at onset also showed tendency to lower levels of C peptide (Table 7). These results support the view that an early detection of diabetes immediately followed by a vigorous treatment aiming at rapid normalization of the metabolism may save the function of some beta cells. Further support is offered by the fact that a better average diabetic control, i.e. higher index figure, was reported for patients with onset after 1970, which coincides in time with an increase in treatment intensity. A previous study from our department including a greater number of patients with onset after 1970 have shown a significant correlation between the occurrence of postinitial remission and later C peptide level (19). The fact that the same correlation was negative in the present study among patients with onset before 1970 may be due to the possibility that patients who during the 1960's had a postinitial remission period were less intensively supervised and therefore subsequently lost the function of initially saved beta cells. However, as shown in Table 7, the pattern of relationships remains unaltered even when patients without remission are analysed separately.

During the first years after onset of juvenile

diabetes insulin and C peptide serum levels are known to decrease in most patients (1 8 10 21) In the present diabetic population there was only a weak negative correlation be tween C peptide and duration however This may indicate that in those patients in whom the beta cell function has been preserved over the first few years an endogenous insulin pro duction may be present for considerably longer periods

In a previous study including more patients with shorter duration of diabetes a negative correlation was found between insulin anti bodies and C peptide (19) This could not be reconfirmed in the present study but a signifi cantly negative correlation between insulin antibodies and diabetic control was observed in contrast to other reports (6 17) This may mean that insulin antibodies disturb diabetic control by some other mechanism than through their supposed negative effect on the beta cell function (19)

During the postinitial remission period the diabetic control is excellent This may be due to the residual beta cell function as demon strated by measurable C peptide levels Our studies have shown that measurable C peptide may persist even after this period Although the fasting C peptide level does not give in formation about the ability of the beta cells to answer to naturally occurring stimuli (23) it does indicate that there is some beta cell func tion This is also supported by the correlation between C peptide and diabetic control

In conclusion our study seems to indicate that an early diagnosis with a rapid normaliza tion of the metabolism at the onset of juvenile diabetes increases the possibility to preserve some of the endogenous insulin production

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Ketonuria	−	n s	<0 005
Onset after 1970	+	−	<0 02
C peptide			
Age at onset	+	<0 001	<0 001
Insulin at onset	+	<0 01	−
Ketonuria	−	<0 05	−
Remission	−	<0 02	n s
Control			
Insulin at onset	+	<0 02	−
Onset after 1970	+	−	<0 01
Duration	−	<0 01	<0 01
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During the first years after onset of juvenile

Acta Pædiatr Scand 66 185–192 1977

RENAL GROWTH AND FUNCTION IN PATIENTS NEPHRECTOMIZED IN CHILDHOOD

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ABSTRACT Aperia A Broberger O Wikstad I and Wilton P (Department of Paediatrics and Paediatric Radiology Karolinska Institute St Goran s Children s Hospital Stockholm Sweden) Renal growth and function in patients nephrectomized in childhood Acta Paediatr Scand 66 185 1977.—Eight patients nephrectomized in childhood were studied with regard to growth and function of the remaining kidney The age of the patients ranged between 8½ and 31 years and the follow up period was from 1 to 20 years In most of the patients repeated renal size determinations were made on postoperative urograms Normal values for kidney size in childhood are also presented The structural hypertrophy continued for at least three years after nephrectomy and was most pronounced in patients nephrectomized before three years of age When more than three years had passed after nephrectomy the remaining kidney was 35–65 % larger than normal Healthy young adults and children with a previous history of urinary tract infection served as controls for function studies The balance between glomerular and tubular function was well preserved in nephrectomized patients The renal surface area showed the same relation to GFR and to reabsorption of bicarbonate in nephrectomized patients as in controls It is therefore concluded that the increase in kidney function following unilateral nephrectomy is at least in early life primarily due to structural enlargement

KEY WORDS Nephrectomy renal hypertrophy kidney size renal function

It has been known for decades that when one kidney is removed compensatory changes occur in the remaining kidney (1 25) The mechanism of this process remains unknown (18) and many questions need to be resolved In the rat which is the most commonly studied species unilateral nephrectomy results in structural enlargement (12 22 24) as well as in increased function (12 24) of the contralateral kidney However there appears to be some dissociation between structural and functional changes (12) It is therefore uncertain to what extent increased renal function is due to structural changes of the nephron and to what extent increased function is secondary to increased blood perfusion relative to the size of the nephron It seems likely that increased function based on structural enlargement provides optimal conditions for complete preservation of renal function On the other hand increased function that is due to increased nephron perfusion might disrupt the normal relationship between glomerular perfusion glomerular filtration and tubular reabsorption which in turn would effect homeostatic efficiency of the kidney

This study concerns the relationship between renal size and renal function in nephrectomized man The function studies include determinations of both glomerular filtration rate (GFR) and tubular reabsorptive capacity Only patients nephrectomized in childhood

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Submitted June 21 1976
Accepted Sept 15 1976

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Table 1 *Clinical data of patients studied*
UTI = urinary tract infection

Patient	Sex	Age at nephrectomy	Symptoms	Kidney disease	Age at the time of study (y)
BL	M	6 m	Fever abdominal tumor	Hypoplastic kidney with no *normal tissue*	12
MR	F	9 m	UTI	Non obstructive hydronephrosis with large reflux	11
MK	M	2 y	Fever abdominal tumor	Hydronephrosis with obstruction in pelvoureteral junction	9½
ML	M	3 y	Recurrent fever UTI	Pyelonephritic kidney with tissue changes indicative of dysplasia	12
LH	F	7½ y	Recurrent UTI	Several cysts in a normal cortex Medulla fibrotic with much primitive tissue	8½
UE	F	8 y	Recurrent UTI 4 years	Polycystic renal dysplasia	19
KL	F	10½ y	Traumatic rupture	Normal kidney	31
EK	F	13 y	*Recurrent back pain*	Hydronephrosis with obstruction in pelvoureteral junction	29

have been included since experimental studies suggest that structural hypertrophy differs in growing and adult animals (9 17)

MATERIAL

Eight patients five females and three males have been studied The age of the patients ranged between 8½ and 31 years Only patients with a normal remaining kidney at nephrectomy were included in this study The time that elapsed after the nephrectomy ranged between 1 and 20 years Details on the patients studied are given in Table 1 In all but one patient (K L) nephrectomy was carried out because of unilateral renal disease The parenchyma of the nephrectomized kidney was therefore generally reduced (Fig 1) At the time of the study all of the patients were in good general condition Three patients developed a urinary tract infection after surgery but not during the two years prior to the study The arterial blood pressure was normal The length and weight of the patients are presented in Fig 2 In no case did nephrectomy influence subsequent length and weight Informed consent for this study was obtained from all of the adult patients and from the parents of the children

Normal renal size was determined in 37 patients ranging in age from 2 months to 30 years All of the patients were referred for urography because of abdominal pain except for infants with a twin who had a malformation None of the patients in this group had a previous history of renal disease The length and weight of the patients were within ±2 S D of normal

Four girls 7 to 13 years old with recurrent urinary tract infection (UTI) but with normal GFR and normal response to an oral ammonium chloride load were used as a control group for the renal function study These patients have been described in a previous report from this laboratory (2)

METHODS

Determination of kidney size The size of the kidney was determined on urograms Five patients were followed with repeated urographies one to five times postoperatively for at least three years In four patients preoperative urograms were available Urography was carried out with the standard technique of anterior posterior and oblique views External ureteral compression was used in each case 0 5–1 ml/kg body weight of contrast medium (60% Urografin Scheering AG) was injected intravenously A film focus distance of 100 cm was used in all cases The size of the renal parenchyma was determined by tracing the outlines of the kidney on transparent paper The renal pelvis was not included in the tracing The traced area was measured with a planimeter All values were correlated to body surface

Renal function This study was performed in the Paediatric Department of St Goran s Hospital The children were hospitalized for one week and received a special diet containing 100–150 mmol Na/1 73 m² body surface area (BSA)/day The adults were studied as outpatients and they were instructed to follow a regular diet without excessive salt At the time of the renal function study blood samples were obtained for determination of Hb WBC SR serum sodium potassium calcium phosphate pH P_{CO_2} and bicarbonate The values were consistently normal Urine cultures before and two weeks after the renal function study were negative In all patients except

Fig 1 Intravenous urogram of a seven year old girl (L. H.) showing a hypoplastic right kidney. The left kidney is slightly hypertrophied.

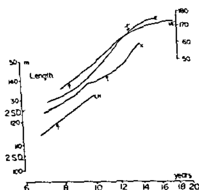


Fig 2a Increase in length of the patients studied. The arrows indicate the age at which nephrectomy was performed. The interrupted lines show the outer limits (±2 S.D.) for normal growth in Swedish children.

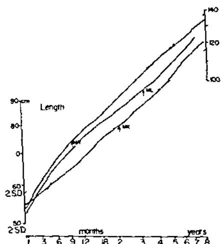


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Table 1 *Clinical data of patients studied*
UTI=urinary tract infection

Patient	Sex	Age at nephrectomy	*Symptoms*	Kidney disease	Age at the time of study (y)
BL	M	6 m	Fever abdominal tumor	Hypoplastic kidney with no normal tissue	12
MR	F	9 m	UTI	Non obstructive hydronephrosis with large reflux	11
MK	M	2 y	Fever abdominal tumor	Hydronephrosis with obstruction in pelvoureteral junction	9½
ML	M	3 y	Recurrent fever UTI	Pyelonephritic kidney with tissue changes indicative of dysplasia	12
LH	F	7½ y	Recurrent UTI	Several cysts in a normal cortex Medulla fibrotic with much primitive tissue	8½
UE	F	8 y	Recurrent UTI 4 years	Polycystic renal dysplasia	19
KL	F	10½ y	Traumatic rupture	Normal kidney	31
EK	F	13 y	Recurrent back pain	Hydronephrosis with obstruction in pelvoureteral junction	29

have been included since experimental studies suggest that structural hypertrophy differs in growing and adult animals (9 17)

MATERIAL

Eight patients five females and three males have been studied The age of the patients ranged between 8½ and 31 years Only patients with a normal remaining kidney at *nephrectomy were included in this study* The time that elapsed after the nephrectomy ranged between 1 and 20 years Details on the patients studied are given in Table 1 In all but one patient (K L) nephrectomy was carried out because of unilateral renal disease The parenchyma of the nephrectomized kidney was therefore generally reduced (Fig 1) At the time of the study all of the patients were in good general condition Three patients developed a urinary tract infection after surgery but not during the two years prior to the study The arterial blood pressure was normal The length and weight of the patients are presented in Fig 2 In no case did nephrectomy influence subsequent length and weight Informed consent for this study was obtained from all of the adult patients and from the parents of the children

Normal renal size was determined in 37 patients ranging in age from 2 months to 30 years All of the patients were referred for urography because of abdominal pain except for infants with a twin who had a malformation None of the patients in this group had a previous history of renal disease The length and weight of the patients were within ±2 S D of normal

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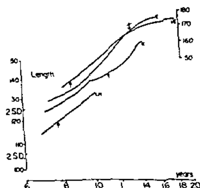


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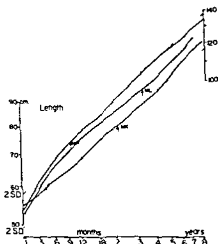


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Fig 5 Intravenous urogram in a nine year old boy (M K) showing compensatory hypertrophy of the left kidney seven years after nephrectomy of the right kidney

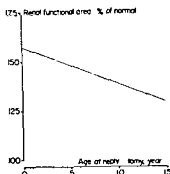


Fig 6 The relationship between size of the remaining kidney 1–11 years postoperatively and age at the time of nephrectomy

HCO_3 The renal HCO_3 threshold was within 2 S D of normal When renal HCO_3 reabsorption was related to the increasing serum HCO_3 value the range of values did not deviate from that found in children with recurrent UTI (Fig 8)

The relationship between renal size and function is shown in Fig 9 and Table 2 The relationship between renal size and GFR has previously been determined in this laboratory in children with recurrent UTI and with and without vesicoureteral reflux GFR was then determined unilaterally The regression line found in that study is shown in Fig 9 The

Table 2 *Renal functional data*

	GFR 1/h/1 73 m BSA	HCO Threshold (mmol/l)	T_{HCO_3} at serum HCO_3 29–31 5 (mmol/l)	Renal surface area 1 73 m² BSA m²×10⁻	GFR Renal surface area	T_{HCO_3} Renal surface area
MR	4 6	6 7	28 4	90	0 052	26
MK	5 46	?3 4	?8 7	110	0 050	?0
ML	4 74	3 0	30 6	9?	0 05?	?3
LH	4 50	? 9	7 5	88	0 051	24
UE	5 10	3 8	8 4	85	0 060	29
KL	4 08	3 0	7 6	90	0 046	16
EK	5 70	4 6	?6 1	–	–	–
BL	5 10	3 5	?8 2	–	–	–
Mean	4 9?	?3 9	?8 2	9? 5	0 05?	?3
S D	0 53	1 3	1 3	8 9	0 005	2
Girls with UTI	6 36±1 0?	4 5±0 9	6 7±0 8	–	0 053±0 008	?0±4
p	<0 001	0 4>p>0 3	0 1>p>0 05		0 8>p>0 7	0 4>p>0 3

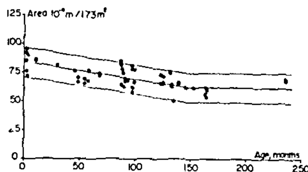


Fig 3 The surface area of one kidney in 0–25 year old females and males The outer lines include ±2 S D

one a urogram was obtained 2 to 14 days before or after the renal function study

Renal function was evaluated with a clearance technique including bladder catheterization and continuous infusion of inulin (Laevasar Gesellschaft) which was the indicator substance for glomerular filtration The study was carried out using a standardized fluid intake of approximately 0 7%/kg body weight every hour Urine was collected during 20 min periods In the middle of each period venous blood samples were withdrawn for analysis of inulin and electrolytes and prewarmed capillary blood samples were taken for determination of pH HCO_3 concentration and Pco_2 pH and bicarbonate were determined in anaerobic urine samples Urine samples were analyzed with the Astrup technique as described previously (2) After two to three control periods an intravenous infusion of 0 6 molar sodium bicarbonate was started with a stepwise increase of infusion rate After 11 to 16 periods when approximately 6 ml/kg bicarbonate had been infused the serum bicarbonate concentration had reached 30–32 mmol/l and the test was interrupted The net fluid expansion during the test averaged 2 2±0 7% of body weight

ANALYTICAL METHODS

Analyses of inulin in serum and urine were made with the anthrone method (15) Sodium concentration in serum and urine was determined with a flame photometer Blood pH bicarbonate and P_{CO_2} were determined with the Astrup method The pH of fresh blood and urine samples were determined (after equilibration with 4% and 8% carbon dioxide gas mixtures) in a pH meter 27 (Radiometer) The bicarbonate concentration of urine was calculated by using the Henderson Hasselbalch equation assuming the pH of urine to be 6 33–0 5 $\sqrt{B}$ where B represents the sum of urinary sodium and potassium concentration in mols/l For details see previous report (2) For statistical analyses the Student s t test has been used

RESULTS

Fig 3 shows the normal relationship between renal surface area and age Renal surface in relationship to body surface area falls slightly but significantly during the first 12 years of life The relationship can be described by the equation (83 70–0 1475X) (X=age in months) After the age of twelve years renal surface in relation to body surface remains constant (62±6)×10^{-4} m²/1 73 m² BSA (mean ±S D)

Renal surface area in nephrectomized patients has been expressed in % of the surface area expected for one kidney in healthy individuals of corresponding age (Fig 4) Preoperatively the healthy kidney was enlarged 107–126% of normal in patients with unilateral renal disease Following unilateral nephrectomy the healthy kidney showed a further increase in size Enlargement of the remaining kidney continued two to four years after the operation After that time the size of the renal parenchyma ranged between 135–164% of the expected size The increase in renal surface *area that occurred was the result of a homogenous* enlargement of the renal parenchyma (Fig 5) The final size of the remaining kidney appears to be inversely related to the age at which nephrectomy is performed (Fig 6)

The data from the renal function studies are summarized in Table 2 The GFR averages 4 92 l/h/1 73 m² BSA This is approximately 75% of the GFR found in healthy young adults (3) with both kidneys intact or 149% of the GFR in one normal kidney The GFR appears to be independent of the present age at this study as well as of the age at nephrectomy (Fig 7*a* and *b*) Renal tubular function was evaluated by studying the reabsorption of

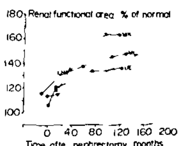


Fig 4 Postoperative increase in renal size determined on repeated urograms in five patients

Previous studies on renal size in nephrectomized man generally include only one value for each individual In this investigation the development of compensatory renal growth has been followed for the first time In all patients who were nephrectomized because of unilateral renal disease the contralateral kidney was 7–26% larger than normal at the time of surgery Removal of the diseased kidney initiated a further relative increase in renal size that continued for at least three years The finding that compensatory structural changes took place over such a long period of time was somewhat unexpected In man functional adaption to unilateral nephrectomy is generally considered to be almost complete after two weeks (11) More recent studies however show that a slight further increase can occur and that this increase is dependent on the age at which nephrectomy is carried out (4)

The size of the hypertrophied kidney at the last determination was 135 to 165% of normal In previous studies of kidney donors the size of the remaining kidney has generally not been more than 25% larger than normal (5 20) In most of those studied however the time from operation to the recording of kidney size has been shorter than 48 months The fact that renal enlargement is more pronounced in patients in the present study might depend on two factors longer observation time and younger age at which nephrectomy was performed The finding in this study that the final size of the kidney was dependent on the age at nephrectomy indicates that the second factor is also of importance

Functional adaption to nephrectomy appears to be more independent of age than structural adaption When GFR is related to body surface the values recorded in this study are well in accordance with those found in healthy kidney donors (4 19) as well as in nephrectomized children (14 23) *i e* 4 6 l/1 73 m² BSA/h Previous studies on kidney donors (21) as well as on nephrectomized children (23) have shown that the maximal transport capacity for glucose and PAH increase in proportion to the GFR in the remaining kidney This is also true for hydrogen ion excretion (7) Other tubular parameters such as phosphate and uric acid transport (21) have however been found to increase out proportion to the GFR This shows that one cannot predict the tubular changes that will occur following ablation of renal tissue but that each transport system should be analyzed individually In the present study tubular reabsorption of HCO_3 was determined since this function is of major importance for control of acid–base balance and even minor disturbances in tubular HCO_3 transport might have important clinical consequences The capacity to reabsorb HCO_3 at high serum HCO_3 levels was increased in proportion to or just above the GFR This parameter like glucose and PAH transport can be used as a quantitative measurement of proximal tubular function The renal HCO_3 threshold was slightly but not significantly lower in the nephrectomized patients The combined finding of a reduced threshold and normal maximal reabsorptive capacity may be interpreted as a sign of heterogeneity of the nephron population (10) The limited range of the HCO_3 reabsorption values when the serum HCO_3 values *were increased* suggests that even if heterogeneity occurs in the hypertrophied kidney it is not of major importance

The normal relationship between renal size and GFR as well as the relationship between renal size and tubular capacity for bicarbonate reabsorption were well preserved in all of the hypertrophied kidneys studied This makes it appear very likely that in patients nephrectomized in childhood functional adaption will be due mainly to structural enlargement of the kidney More studies are needed to show if this is also true of individuals nephrectomized in adulthood

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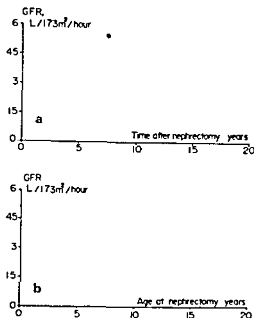


Fig 7a Glomerular filtration rate 1–20 years following nephrectomy
Fig 7b Glomerular filtration rate related to age at ne phrectomy

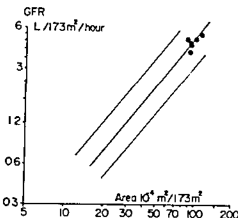


Fig 9 The relationship between GFR and renal surface area The dots represent values obtained in nephrectomized patients The lines represent the mean ±2 S D of values in patients with two kidneys and a past history of urinary tract infection

relationship between renal size and function in nephrectomized patients is also compared to that found in girls with recurrent UTI whose bicarbonate reabsorptive capacity was studied (Table 2) It is apparent from the table and the figure that the relationship between renal size and GFR as well as between renal size and tubular reabsorptive capacity of HCO_3 is the same in nephrectomized patients as in patients with two functioning kidneys

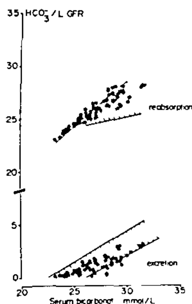


Fig 8 Reabsorbed and excreted bicarbonate at different serum bicarbonate values The dots represent individual values recorded *in nephrectomized patients* The shaded areas show the outer limits for normal values recorded in patient with two kidneys

DISCUSSION

The majority of studies concerning compensa tory hypertrophy have been carried out in rats but this species continues to grow throughout life When renal mass is removed in the adult rat the structural and functional changes are proportional to the mass of tissue removed (16) There exists however some dissociation between structural and functional adaption Some investigators have found that an in crease in GFR precedes the structural enlarge ment (22) When adaptive changes are com pleted three to four weeks after nephrectomy GFR has increased 50–90% (12 15 16) while the increase in kidney weight averages 35–50% (15 16) The increment in renal mass following unilateral nephrectomy is more pro nounced in young rats and also depends more on hyperplasia than hypertrophy of the cells (9) It has also been reported that nephrec tomy in rats younger than 30 days results in increased formation of new nephrons (6) but this has not been confirmed by other investi gators (17)

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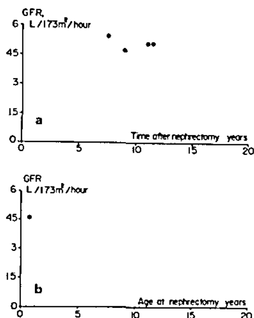


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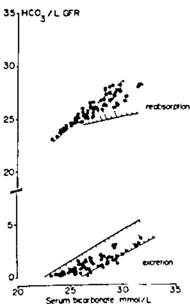


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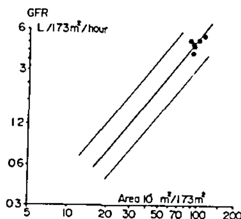


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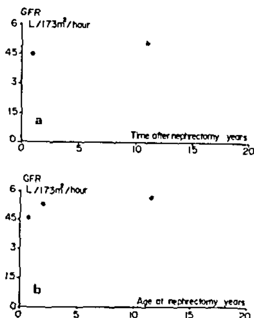


Fig 7a Glomerular filtration rate 1–20 years following nephrectomy

Fig 7b Glomerular filtration rate related to age at nephrectomy

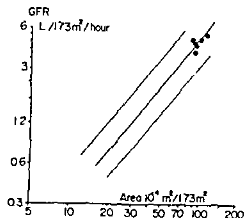


Fig 9 The relationship between GFR and renal surface area The dots represent values obtained in nephrectomized patients The lines represent the mean ±2 S D of values in patients with two kidneys and a past history of urinary tract infection

relationship between renal size and function in nephrectomized patients is also compared to that found in girls with recurrent UTI whose bicarbonate reabsorptive capacity was studied (Table 2) It is apparent from the table and the figure that the relationship between renal size and GFR as well as between renal size and tubular reabsorptive capacity of HCO_3 is the same in nephrectomized patients as in patients with two functioning kidneys

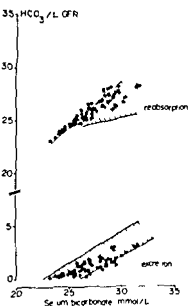


Fig 8 Reabsorbed and excreted bicarbonate at different serum bicarbonate values The dots represent individual values recorded in nephrectomized patients The shaded areas show the outer limits for normal values recorded in patient with two kidneys

DISCUSSION

The majority of studies concerning compensatory hypertrophy have been carried out in rats but this species continues to grow throughout life When renal mass is removed in the adult rat the structural and functional changes are proportional to the mass of tissue removed (16) There exists however some dissociation between structural and functional adaption Some investigators have found that an increase in GFR precedes the structural enlargement (22) When adaptive changes are completed three to four weeks after nephrectomy GFR has increased 50–90% (12 15 16) while the increase in kidney weight averages 35–50% (15 16) The increment in renal mass following unilateral nephrectomy is more pronounced in young rats and also depends more on hyperplasia than hypertrophy of the cells (9) It has also been reported that nephrectomy in rats younger than 30 days results in increased formation of new nephrons (6) but this has not been confirmed by other investigators (17)

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KEY WORDS Infantile periarteritis nodosa mucocutaneous lymph node syndrome coronary artery aneurysm Kawasakis disease

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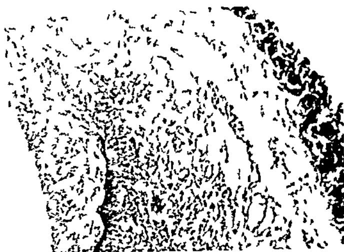


Fig 3 Case 1 Longitudinal section from right coronary artery Widespread inflam mation in all parts of the vessel wall de struction of elastic lamina in the upper part Fibrincoated epicardium to the right H–E ×88

and tortuous (Fig 4) and the proximal part of the left descending branch contained a 1 cm thick thrombus The vessel had ruptured at this site The lungs liver and spleen were heavy and congested

Microscopy disclosed partly destroyed walls of the coronary arteries with widespread acute inflammation with polymorphonuclear leucocytes (Fig 5) In the left ventricular myocardium there were fresh necroses In both iliac arteries and in branches of the mesenteric and the perirenal arteries areas of acute inflammation were present Millimeter wide arteries in the periadrenal fat testis and epididymis showed fibrinoid necrosis of the vessel wall with infiltration of polymorphonuclear leuko-cytes

Case 3

A 4-year-old boy was admitted to hospital in april 1966 be cause of persistent fever sore throat and enlargement of cervical lymph nodes in spite of penicillin and ampicillin treatment for one week A few days later the patient s hands and ankles became swollen and tender Examina tion of the blood revealed a leucocytosis (maximum 000/mm) with a pronounced neutrophilia Urine and CSF analyses were normal Protein electrophoresis re vealed an increased α_2-globuline concentration No bac terial growth was shown in cultures of blood urine CSF and stools No virus could be i olated ECG was initially normal but later deep Q waves and ST T abnormalities ap-peared in the left precordial leads

In the summer of 1966 the heart was normal at roentgen examination In may 1967 the heart volume was moderate ly increased During the autumn the volume decreased somewh t and later a sl ght enlargement of the heart was invariably demonstrated at repeated examinations

The child improved and the fever and arthritis dis appeared after three weeks He was continuously treated with peni illin When discharged from hospital he was in good condition However an abnormal ECG pattern and a card a enlargement persisted

Cardia catheterizatio n and angiocardiography with in jection of contrast medium into the left ventricle was car ried out in 1969 Two aneurysms were demonstrated in the right coronary artery and in the left coronary artery there was an aneurysm at the arterial bifurcation (Fig 6*a*) A few months later the proximal part of the right coronary aneurysms was partly filled with a thrombotic mass and the artery was occluded close to this aneurysm The distal part of the right coronary artery was filled in a retrograde direction from the left circumflex artery

Two years later the right coronary artery was totally occluded close to its origin from the aorta and the aneu rysm of the left coronary artery had increased in size (Fig 6*b*)

The boy is now 13 years old and is in good condition He has no angina pectoris He has been treated with anti coagulants during the past six years



Fig 4 Case 2 Opened heart Right coronary artery (at the left part of the picture) cm wide thin walled tortuous with inbulging ridges



Fig 1 Case 1 Chest examination one day prior to death Heart volume is increased There is a bulge at the left of the heart which was not present at previous examination

obscure origin who all developed aneurysms of the coronary arteries

CASE HISTORIES

Case 1

A previously healthy 6-month old girl was admitted to hospital because of persistent high feber sore throat conjunctivitis signs of meningitis diarrhoea and a maculopapular exanthema over the trunk In addition oedema in the face and on the hands and a moderate hepatomegalia were noticed on admittance to hospital Laboratory examination of the blood revealed leucocytosis (at most 29000/mm³) and marked neutrophilia accelerated erythrocyte sedimentation rate (maximum 70 mm) and positive CRP Serum protein electrophoresis revealed increased α_2 globuline and IgM concentration and a pathological band in the gamma region on immune electrophoresis analysis LDH was slightly elevated Analysis of the cerebrospinal fluid (CSF) revealed 20 mononuclear leucocytes/mm³ and a protein content of 0 50 g/l Urine analysis was without remark Scintigraphy of the liver and the brain were normal EEG was normal Sinus tachycardia was constantly found at ECG which otherwise was normal at repeated examinations Bacterial cultures from the throat nose urine stool and CSF were all negative Complement fixation tests were negative and no virus could be isolated

Normal appearance of heart and lungs was demonstrated at repeated roentgen examinations during the initial period

The infant was treated with different antibiotics (penicillin gentamycin and cephalothin) without any effect on the temperature or the general condition After administration of hydrocortisone (?00 mg/day) the temperature normalized and the infant improved within a few days The fever reappeared when the hydrocortisone treatment was stopped The patient was then given prednisolon orally the temperature decreased the exanthema disappeared and the infant improved However after three weeks treatment the infant died suddenly

On the day prior to death roentgen examination showed that the heart volume had increased substantially and a bulge had appeared on the upper left border of the heart (Fig 1)

Necropsy A small amount of coagulated blood was found in the pericardial sac and a fibrinous pericarditis on the anterior cardiac surface The heart was enlarged mainly due to left ventricular hypertrophy The coronary arteries were profoundly dilated and tortuous (Fig 2) The liver was enlarged and congested

Microscopic examination revealed widespread inflammation of the coronary arteries with oedema and destruction of parts of the arterial wall Polymorphonuclear leucocytes predominated in the exudate (Fig 3) Similar inflammatory changes were also seen in small areas in the carotid and superior mesenteric arteries and in some veins in the lungs and the liver Other organs were normal

Case 2

A 18 month old boy was admitted to hospital because of an obscure exanthema The exanthema disappeared and the boy was sent home

Six weeks later the patient became ill with high fever exanthema and cough scarlet fever was suspected Treatment with penicillin had no effect After a few days desquamation of the skin was noticed on the fingers A moderate fever persisted the appetite was poor and the patient was remarkably tired He died from a sudden cardiac arrest at home

Necropsy A 55 mm thick fresh dark red coagulum surrounded the heart The coronary arteries were dilated

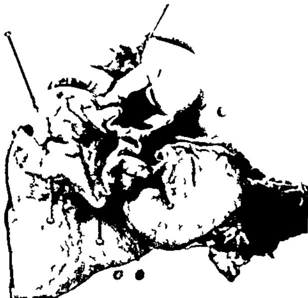


Fig 2 Case 1 Part of the heart with right coronary cut open greatly dilated and tortuous The pericardium is fibrin coated and thickened



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Fig 1 Case 1 Chest examination one day prior to death Heart volume is increased There is a bulge at the left of the heart which was not present at previous examination

obscure origin who all developed aneurysms of the coronary arteries

CASE HISTORIES

Case 1

A previously healthy 6-month old girl was admitted to hospital because of persistent high feber sore throat conjunctivitis signs of meningitis diarrhoea and a maculopapular exanthema over the trunk In addition oedema in the face and on the hands and a moderate hepatomegalia were noticed on admittance to hospital Laboratory examination of the blood revealed leucocytosis (at most 29000/mm³) and marked neutrophilia accelerated erythrocyte sedimentation rate (maximum 70 mm) and positive CRP Serum protein electrophoresis revealed increased α_2 globuline and IgM concentration and a pathological band in the gamma region on immune electrophoresis analysis LDH was slightly elevated Analysis of the cerebrospinal fluid (CSF) revealed 20 mononuclear leucocytes/mm³ and a protein content of 0 50 g/l Urine analysis was without remark Scintigraphy of the liver and the brain were normal EEG was normal Sinus tachycardia was constantly found at ECG which otherwise was normal at repeated examinations Bacterial cultures from the throat nose urine stool and CSF were all negative Complement fixation tests were negative and no virus could be isolated

Normal appearance of heart and lungs was demonstrated at repeated roentgen examinations during the initial period

The infant was treated with different antibiotics (penicillin gentamycin and cephalothin) without any effect on the temperature or the general condition After administration of hydrocortisone (200 mg/day) the temperature normalized and the infant improved within a few days The fever reappeared when the hydrocortisone treatment was stopped The patient was then given prednisolon orally the temperature decreased the exanthema disappeared and the infant improved However after three weeks treatment the infant died suddenly

On the day prior to death roentgen examination showed that the heart volume had increased substantially and a bulge had appeared on the upper left border of the heart (Fig 1)

Necropsy A small amount of coagulated blood was found in the pericardial sac and a fibrinous pericarditis on the anterior cardiac surface The heart was enlarged mainly due to left ventricular hypertrophy The coronary arteries were profoundly dilated and tortuous (Fig 2) The liver was enlarged and congested

Microscopic examination revealed widespread inflammation of the coronary arteries with oedema and destruction of parts of the arterial wall Polymorphonuclear leucocytes predominated in the exudate (Fig 3) Similar inflammatory changes were also seen in small areas in the carotid and superior mesenteric arteries and in some veins in the lungs and the liver Other organs were normal

Case 2

A 18 month old boy was admitted to hospital because of an obscure exanthema The exanthema disappeared and the boy was sent home

Six weeks later the patient became ill with high fever exanthema and cough scarlet fever was suspected Treatment with penicillin had no effect After a few days desquamation of the skin was noticed on the fingers A moderate fever persisted the appetite was poor and the patient was remarkably tired He died from a sudden cardiac arrest at home

Necropsy A 55 mm thick fresh dark red coagulum surrounded the heart The coronary arteries were dilated

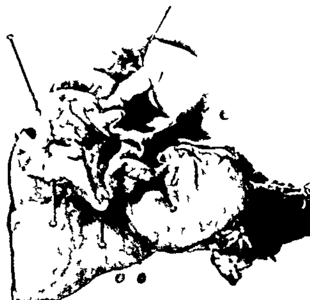


Fig 2 Case 1 Part of the heart with right coronary cut open greatly dilated and tortuous The pericardium is fibrin coated and thickened



Fig 3 Case 1 Longitudinal section from right coronary artery Widespread inflammation in all parts of the vessel wall destruction of elastic lamina in the upper part Fibrincoated epicardium to the right H-E × 88

and tortuous (Fig 4) and the proximal part of the left descending branch contained a 1 cm thick thrombus The vessel had ruptured at this site The lungs liver and spleen were heavy and congested

Microscopy disclosed partly destroyed walls of the coronary arteries with widespread acute inflammation with polymorphonuclear leucocytes (Fig 5) In the left ventricular myocardium there were fresh necroses In both iliac arteries and in branches of the mesenteric and the perirenal arteries areas of a ute inflammation were present Millimeter wide arteries in the periadrenal fat testis and epididymis showed fibrinoid necrosis of the vessel wall with infiltration of polymorphonuclear leukocytes

Case 3

A 4-year-old boy was admitted to hospital in april 1966 because of persistent fever sore throat and enlargement of cervical lymph nodes in spite of penicillin and ampicillin treatment for one week A few days later the patient s hands and ankles became swollen and tender Examination of the blood revealed a leucocytosis (maximum 000/mm) with a pronounced neutrophilia Urine and CSF analyses were normal Protein electrophoresis revealed an increased α globuline concentration No bacterial growth was shown in cultures of blood urine CSF and stools No virus could be isolated ECG was initially normal but later deep Q waves and ST T abnormalities appeared in the left precordial leads

In the summer of 1966 the heart was normal at roentgen examination In may 1967 the heart volume was moderately increased During the autumn the volume decreased somewhat and l ter a slight enlargement of the heart was invariably demonstrated at repeated examinations

The child improved and the fever and arthritis disappeared after three weeks He was continuously treated with pen cillin When di charged fr m hospital he was in good conditi n H wever an abnormal ECG pattern and a card ac enlargement persisted

Cardiac catheterization and angiocardiography with injection of contrast medium into the left ventricle was carried out in 1969 Two aneurysms were demonstrated in the right coronary artery and in the left coronary artery there was an aneurysm at the arterial bifurcation (Fig 6*a*) A few months later the proximal part of the right coronary aneurysms was partly filled with a thrombotic mass and the artery was occluded close to this aneurysm The distal part of the right coronary artery was filled in a retrograde direction from the left circumflex artery

Two years later the right coronary artery was totally occluded close to its origin from the aorta and the aneurysm of the left coronary artery had increased in size (Fig 6*b*)

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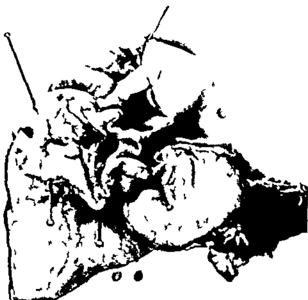


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Table 1 *Common clinical features in infantile periarteritis nodosa (IPN) and the mucocutaneous lymph node syndrome (MLNS) compared with symptoms and signs in the present cases*

	IPN	MLNS	Present cases 1	2	3	4
Prolonged fever not responding to antibiotics	+	+	+	+	+	+
Congestion of ocular conjunctive	+	+	+	?	−	+
Lesions of the lips and oral cavity	+	+	+	+	+	+
Changes in hands and feet	+	+	+	+	+	−
Polymorphous exanthema	+	+	+	+	?	+
Acute nonsuppurative swelling of cervical lymph nodes	+	+	+	?	+	+
Cardiovascular signs	+	+	+	+	+	+
Diarrhoea	+	+	+	−	−	−
Proteinuria	+	+	+	−	−	−
Leucocytosis with neutrophilia	+	+	+	?	+	+
Increased ESR	+	+	+	?	+	+
Positive CRP	+	+	+	?	?	
Increased serum α_2-globulin	+	+	+	?	+	?
Negative AST	+	+	+	?	+	+

Necropsy The pericardium contained 300 ml clotted blood The main branches of the left coronary artery were widely almost aneurysmatically dilated but narrowed gradually towards the heart apex The anterior descending branch contained a large thrombus The myocardium was normal the lungs were heavy and oedematous and a small amount of transsudate was present in the pleural sac

Microscopically the walls of the coronary artery were largely destroyed by widespread inflammation

DISCUSSION

The description in the literature of the clinical history of IPN and MLNS are identical in most respects (2 4 5 8 13 14 15 18 20 21 22) The present four cases share many characteristic symptoms with both IPN and MLNS (Table 1) The fatal outcome in three of the children was quite unexpected It seems impossible to differentiate between IPN and MLNS on clinical and laboratory grounds However the prognosis of the two diseases is considered to differ profoundly

According to Kawasaki et al (13) the pathological findings in MLNS and IPN are identical they differ however in their clinical evolution The authors added that the clinical picture of IPN has got only scant descriptions

The necropsy findings in our cases are almost identical to those previously reported in both IPN and MLNS Thus not even the histological examination can differentiate the two conditions

In the original description of polyarteritis nodosa by Kussmaul & Maier (16) it was stated that the disease affected medium sized arteries mainly in the splanchnic area Similar changes were noted by Arkin (1) Since then varying arterial changes have been described as polyarteritis nodosa including changes in fairly small arteries detectable at histological examination only Clinical investigations and animal experiments led Zeek et al (25 26) to set up more clearly defined criteria on polyarteritis nodosa They stated that only medium sized arteries should be affected and arteritis not fulfilling the criteria should be designed as hypersensitivity angiitides According to Zeek s criteria our cases represent polyarteritis nodosa Current textbooks however have wider and more varying criteria of polyarteritis nodosa

The etiology of periarteritis nodosa and MLNS is unknown It is notable that rickettsia like bodies have been found in biopsy specimens from patients with MLNS (9) as Treponema pallidum and rickettsia are the



Fig 5 Case 2 Transverse section from middle part of left descending coronary artery Greatly widened artery with inflammation spreading into the surrounding fat Parts of the media preserved in the lower middle otherwise destroyed wall H-E × 88

Case 4

A previously healthy 9 year old boy was admitted to hospital because of exanthema and continuous high fever in spite of a four days treatment with ampicillin At admission he was remarkably tired had a stiff neck conjunctivitis swollen and red tonsils and a fine papulous exanthema A few days later a strawberry tongue was noticed

Analysis of the blood showed leucocytosis (14 000/ mm³) and a neutrophilia The sedimentation rate was 81 mm/hr Analysis of CSF revealed 16 leucocytes/mm³ Urine analysis was normal Bacterial cultures from the urine and throat were negative ECG and roentgen examination of the chest were normal

The boy was treated with penicillin and meticillin but the high fever persisted He had no signs of cardiac failure Nine days after admission to hospital he was found dead in bed



Fig 6 (*a*) Case 3 Left ventricular angiocardiography performed at the age of 8 years The right coronary artery is wide and is the site of two aneurysms (arrows) In the left coronary artery there is an aneurysm at the bifurcation (*b*) Supravalvular aortography performed at the age of 12 years The right coronary artery is now totally occluded 5–6 mm from where it branches from the aorta Proximally to the occlusion small branches appear supplying the right ventricle The left sided aneurysm has increased in size

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Positive CRP	+	+	+	?	?	?
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Negative AST	+	+	+	?	+	+

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cause of other types of arteritis e g syphilis and Rocky Mountain spotted fever

In the light of the general experience that coronary aneurysm is a very rare disease in childhood we find the accumulation of such cases in a small area of Sweden with about 2 million inhabitants exceptional It is also remarkable that so few reports have appeared from other parts of the world since the original report of Kawasaki (3 10 17 19 23) This may partly be due to ignorance of the disease and the uncharacteristic and obscure symptoms

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Submitted Sept 16 1976
Accepted Sept 28 1976

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Acta Pædiatr Scand 66 199–204 1977

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KEY WORDS Cystic fibrosis arginine glucagon

It has been recognized for some time that there is an increased incidence of diabetes mellitus in cystic fibrosis (21) Diminished insulin output in cystic fibrosis in response to oral and intravenous glucose has been reported by Milunsky (19) Milner (18) and Handwerger (9) No absolute cause has been found to account for the insulinopenia Several possibilities have been suggested Want (26) postulated that the gene for cystic fibrosis and diabetes are linked Charles & Kelley (6) in 1961 suggested that the excretory cell defect seen in cystic fibrosis also occurs in the beta cells Handwerger (9) thought the insulinopenia was due to destruction of the islets of Langerhans by pancreatic fibrosis or architectural disorganisation The possibility of a defect in the entero insular axis was considered by Milner (18) in 1969 Several publications recently would suggest that glucagon may be implicated in the aetiology of diabetes mellitus (4 24)

In order to see if glucagon secretion had any hand to play in the increased incidence of diabetes in cystic fibrosis or if the glucagon output was also diminished we investigated children with cystic fibrosis using arginine intravenously as a stimulus to both alpha and beta cells

METHODS

Seventeen children with cystic fibrosis aged between 2 and 17 years mean age 6 4 years diagnosed by clinical signs and by elevated sweat electrolytes (pilocarpine

cause of other types of arteritis e g syphilis and Rocky Mountain spotted fever

In the light of the general experience that coronary aneurysm is a very rare disease in childhood we find the accumulation of such cases in a small area of Sweden with about 2 million inhabitants exceptional It is also remarkable that so few reports have appeared from other parts of the world since the original report of Kawasaki (3 10 17 19 23) This may partly be due to ignorance of the disease and the uncharacteristic and obscure symptoms

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Submitted Sept 16 1976
Accepted Sept 8 1976

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Acta Pædiatr Scand 66 199–204 1977

INSULIN AND GLUCAGON RESPONSE TO ARGININE INFUSION IN CYSTIC FIBROSIS

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ABSTRACT Redmond A O B Buchanan K D and Trimble E R (The Royal Belfast Hospital for Sick Children Belfast and The Department of Medicine Queen s University Belfast) Insulin and glucagon response to arginine infusion in cystic fibrosis Acta Paediatr Scand 66 199 1977 —The glucagon and insulin responses to intravenous arginine were studied in 17 children with cystic fibrosis and in 9 control children It was found that the overall secretion of insulin was diminished however four of the CF children did have a normal output The glucagon responses did not parallel those of insulin The glucagon output varied in the CF children—seven had normal four excessively high and six low output Three of the four children with extremely high output had more severe disease and were below the third centile on the weight chart These four had a fasting hypoglycaemia and also a very low glucose and insulin response We have confirmed diminished insulin secretion in cystic fibrosis but diminished glucagon secretion was only noted when some insulin secretion was preserved The high levels of glucagon seen in the most insulin deficient subjects may be derived from extrapancreatic sources or may be associated with stress reaction in these patients who also had most severe pulmonary involvement The data would be consistent with diminished glucagon and insulin secretion from the pancreas but as the disease progresses an excessive secretion of extra pancreatic glucagon results

KEY WORDS Cystic fibrosis arginine glucagon

It has been recognized for some time that there is an increased incidence of diabetes mellitus in cystic fibrosis (21) Diminished insulin output in cystic fibrosis in response to oral and intravenous glucose has been reported by Milunsky (19) Milner (18) and Handwerger (9) No absolute cause has been found to account for the insulinopenia Several possibilities have been suggested Want (26) postulated that the gene for cystic fibrosis and diabetes are linked Charles & Kelley (6) in 1961 suggested that the excretory cell defect seen in cystic fibrosis also occurs in the beta cells Handwerger (9) thought the insulinopenia was due to destruction of the islets of Langerhans by pancreatic fibrosis or architectural disorganisation The possibility of a defect in the entero insular axis was considered by Milner (18) in 1969 Several publications recently would suggest that glucagon may be implicated in the aetiology of diabetes mellitus (4 24)

In order to see if glucagon secretion had any hand to play in the increased incidence of diabetes in cystic fibrosis or if the glucagon output was also diminished we investigated children with cystic fibrosis using arginine intravenously as a stimulus to both alpha and beta cells

METHODS

Seventeen children with cystic fibrosis aged between ? and 1? years mean age 6 4 years diagnosed by clinical signs and by elevated sweat electrolytes (pilocarpine

Table 1 *Glucagon insulin and glucose response in cystic fibrosis children and controls following iv arginine*

Sex	Age (yrs)	Height (centile)	Weight (centile)	Glucagon response	Pulmonary disease
Cystic fibrosis					
M	2	50	50	Low	Mild
M	2	50	50	Low	Mild
M	5	50	50	Low	Mild
F	6	75	50	Low	Mild
M	6	10	50	Low	Mild
M	6	30	50	Normal	Mild
M	7	75	50	Normal	Mild
M	10	75	50	High	Mild
M	5	50	25	Normal	Moderate
M	7	25	25	Normal	Moderate
F	9	30	25	Low	Moderate
M	12	50	50	Normal	Moderate
F	4	3	<3	High	Severe
F	5	25	3	High	Severe
M	6	50	3	High	Severe
M	7	75	10	Normal	Severe
F	9	10	3	Normal	Severe
Controls					
M	3	25	75	–	–
M	6	10	10	–	–
M	7	75	25	–	–
F	7	10	10	–	–
F	9	25	75	–	–
F	9	90	50	–	–
M	9	10	10	–	–
F	10	90	90	–	–
F	10	25	10	–	–

iontophoresis) were studied and compared with nine control children aged between 3 and 10 years mean age 7 8 years Informed consent was obtained from the parents of all the children studied The control children were healthy children who had recovered from a minor illness All the children were tested after an overnight fast A polythene cannula was inserted in an arm vein and a fasting blood sample for blood glucose insulin and glucagon taken An infusion of 10% i arginine hydrochloride 0 5 g/kg body weight was given over a 30 min period Fifteen minutes after the start of the infusion a second sample was taken and again at 30 45 and 60 min The blood was placed in pre-cooled heparinized tubes at 4 C and transported in ice to the laboratory where it was centrifuged at 4 C Plasma glucose was measured by a micro technique (22) Insulin and glucagon were measured by radioimmunoassay

Radioimmunoassay technique

The plasma for insulin was kept frozen at −20 C until assay The assay could detect 0 5 U/ml and used a single antibody and charcoal separation technique

Glucagon assay

Blood samples for glucagon assay were taken into chilled heparinized tubes centrifuged at 4 C and the plasma extracted by the method of Heding (10) The extracts were reconstituted prior to assay in phosphate buffer pH 7 4 0 04 M Glucagon ^{125}I was prepared according to the method of Jorgensen & Larsen (11) The separation method used was serum and dextran-coated charcoal (3) Two antibodies raised to pancreatic glucagon were used in the glucagon assay viz YY89 at a final dilution of 1 45000 and YY57 at a final dilution of 1 22500 YY89 reacts with the C terminal region of glucagon and YY57 with the N terminal portion of glucagon (8) YY89 in classical terminology has been considered to be pancreas specific and YY57 to be non specific and measure total glucagon like immunoreactivity (GLI) However we have found that some species of gut GLI react with YY89 (8) and some species of pancreatic GLI do not react with YY89 (7) For this reason we have departed from the usual terminology of pancreatic glucagon and total GLI and refer to the material measured by YY89 as C terminal GLI (C-GLI) and that by YY57 as N terminal GLI (N GLI) This takes account of the lack of knowledge concerning the forms of GLI in the tissues and circulation The assays can detect between 10 and 20 pg/ml of glucagon No cross reaction has been noted with other gut and Islet hormones including insulin (Human MRC Standard) gastric inhibitory polypeptide and motilin (both gifts from J Brown) cholecystokininpancreozymin and vasoactive intestinal peptide (both gifts from V Mutt) and human synthetic gastric (ICI) The assay has been reported in detail elsewhere (5)

For simplicity we will henceforth refer to our measurement of C-GLI as glucagon (Only values of glucagon measured by the C terminal reactive antibody being given)

RESULTS

In the nine control children the infusion of arginine caused a rise of plasma glucagon and insulin in 15 min reaching a maximum at 30 min The mean glucagon (Table 2) fasting level of 34 pg/ml rose to a maximum of 246 pg/ml All the children demonstrated a rise greater than 100 pg/ml Insulin rose in all the control children from a fasting mean of 3 µU/mol to a peak of 55 µU/ml at 30 min The mean fasting glucose concentration increased from 71 mg/100 ml by an average increase of 20 mg/100 ml

Considering the 17 cystic fibrosis children as a group the blood sugar response closely parallels the rise seen in the control group with mean peak at 30 min being 85 mg/100 ml The rise of plasma glucagon in the cystic fibrosis group was similar to the controls but the cystic fibrosis group showed a smaller insulin response (mean peak value 22 µU/ml) How

Table 2 *Glucagon insulin and glucose response in cystic fibrosis children and controls following i v arginine*

Mean±S D

Time	0	15	30	45	60
Cystic fibrosis					
Glucagon pg/ml	154±44	277±97	363±113	271±84	203±91
Insulin μU/ml	3±1	14± 5	22±12	8± 4	2± 1
Glucose mg/100 ml	71± 4	85± 5	85± 6	80± 6	70± 5
Control					
Glucagon pg/ml	34±19	177±23	246±58	81±27	60±23
Insulin μU/ml	3± 1	28± 7	55±16	14± 6	12± 6
Glucose mg/100 ml	71± 2	96±10	91± 4	68± 3	58± 1

ever four of the children had a response within normal range

When the glucagon results from the cystic fibrosis patients were analysed separately it appeared that there were three different patterns of response—high normal and low (Fig 1) (High demonstrated a rise greater than 600 pg/ml normal response was greater than 100 pg/ml and low was less than 100 pg/ml)

There were six children in the low response group seven in the normal and four in the high response group In the low response group the mean insulin response was not significantly different from the control group The blood sugar closely followed the control response the only significant difference being at 45 min level In the normal group the glucagon pattern closely followed the controls but there was significant differences in 45 min level ($p<0.05$) The insulin secretion was significantly lower than the controls at 30 min ($p<0.05$)

The third group the high glucagon responders presented interesting findings Of the four children in this group three were classified as having severe lung disease with exocrine pancreatic deficiency while the fourth child had moderate disease and no clinical evidence of malabsorption The mean fasting glucagon level was considerably elevated at 409 pg/ml and the level rose to a maximum of >1 085 pg/ml at 30 min the difference from the controls being significant at all times ($p<0.0025$) The insulin output in contrast was low with significant differences from controls at 30 min ($p<0.05$)

In view of the low glucose response in these four children we considered the possibility that the glycogen stores in the liver were depleted Two of the children in this group were given i v glucagon and an adequate rise in blood sugar was obtained (Table 3)

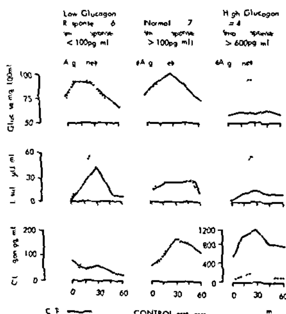


Fig 1 Glucose insulin and glucagon response following i v arginine infusion in control and C F children (C F children are divided into 3 groups according to glucagon response)

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excessive secretion which may be from extrapancreatic sources It is possible that depletion of glucagon and insulin within the pancreas may act as a stimulus to the secretion of extra pancreatic glucagon A recent study by Kalk et al (13) has shown elevated fasting glucagon levels and increased responses to intravenous tolbutamide in subjects with severe chronic pancreatitis

In addition excessive secretion of extra pancreatic glucagon is usually not seen unless there is associated insulin deficiency such as in the studies by Kalk et al (12) in patients with chronic pancreatitis and in the studies by Vranic et al (25) and Matsuyama & Foa (16) in dogs Our results would be entirely consistent with this in that it was only the most severely insulin deficient children who had such excessive glucagon responses whereas the children with normal insulin secretion had defective glucagon secretion Our results would be consistent with the conclusions that secretion of insulin and glucagon from the pancreas may be deficient in children with cystic fibrosis but when the insulin deficiency becomes severe high glucagon levels may occur the source of this glucagon however may be extra pancreatic

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Table 3 *Glucose response following i v glucagon in two children who had a poor glycaemic response after i v arginine*

Glucose rise with arginine (mg/100 ml)	Glucose levels following i v glucagon (mg/100 ml) Minutes after injection		
	0	5	10
7	80	93	109
3	70	105	114

DISCUSSION

Stahl et al (23) demonstrated a diminished insulin and glucagon output in response to arginine in children with cystic fibrosis favouring the hypothesis that the endocrine part of the pancreas in cystic fibrosis is involved in a similar process to that affecting the exocrine cells

We found an overall diminished output of insulin in the 17 children studied 4 of them did have a normal output The glucagon response was variable The basal fasting glucagon level was higher in the children with cystic fibrosis and the response to arginine varied from very low to excess The most interesting group were four children who had a very high glucagon response following arginine and also had a high fasting glucagon level This was associated with low fasting blood sugar the glucose and insulin responses to arginine being very low It is possible that the high glucagon response could be secondary to hypoglycaemia (15) All children studied were fasted for a similar period and in view of the apparently normal glycogen stores and the normal response to glucagon in two of the children in this particular group we are unable to explain the hypoglycaemia

Porte et al (20) have shown low circulating insulin levels following epinephrine infusion High circulating catecholamines have been reported by Barbero & Braddock (1) in cystic fibrosis We considered this a possible reason for the insulinopenia However Handwerger et al (9) found that an infusion of phentolamine given two hours after a GTT caused no change in the insulin level Hence it is unlikely that a raised circulating catecholamines could account for the low insulin/glucagon ratio in these four children Raised glucagon levels have been found in certain states associated with increased metabolic rate such as following burns (27) and trauma (17) Two other conditions associated with high glucagon levels are liver cirrhosis (14) and renal failure (2) but there was no evidence to suggest the presence of either of these two conditions in the cystic fibrosis patients studied here

Analogous results have been described in chronic pancreatitis (12) in association with low insulin response

At the present time we cannot state with certainty the sources of the circulating glucagon which are measuring by radioimmunoassay Our pancreatic glucagon antiserum reacts with the C Terminal portion of the glucagon molecule and this has been thought to be associated with a pancreatic glucagon specific antiserum This antiserum has low cross reactivity with extracts of gut but recent studies from our laboratory would suggest that there are some materials particularly in the stomach and colon that will cross react with this antiserum (7) In addition studies in dogs have suggested that the proximal gastrointestinal tract contains material very similar to pancreatic glucagon and indeed circulating pancreatic glucagon has been found to rise following pancreatectomy (16 25)

It is therefore possible that the glucagon levels which we are recording in this study especially in the insulinopenia patients are being derived from extra pancreatic sources Unless glucagon radioimmunoassays are modified and improved to recognize this the question will remain unanswered

We can conclude that insulin secretion is depleted in the majority of patients with cystic fibrosis However the conclusions with regard to glucagon secretion must remain guarded Some children do have diminished glucagon secretion but others have apparently

excessive secretion which may be from ex trapancreatic sources It is possible that deple tion of glucagon and insulin within the pancreas may act as a stimulus to the secretion of extra pancreatic glucagon A recent study by Kalk et al (13) has shown elevated fasting glucagon levels and increased responses to in travenous tolbutamide in subjects with severe chronic pancreatitis

In addition excessive secretion of extra pancreatic glucagon is usually not seen unless there is associated insulin deficiency such as in the studies by Kalk et al (12) in patients with chronic pancreatitis and in the studies by Vra nic et al (25) and Matsuyama & Foa (16) in dogs Our results would be entirely consistent with this in that it was only the most severely insulin deficient children who had such exces sive glucagon responses whereas the children with normal insulin secretion had defective glucagon secretion Our results would be con sistent with the conclusions that secretion of insulin and glucagon from the pancreas may be deficient in children with cystic fibrosis but when the insulin deficiency becomes severe high glucagon levels may occur the source of this glucagon however may be extra pan creatic

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Stahl et al (23) demonstrated a diminished insulin and glucagon output in response to arginine in children with cystic fibrosis favouring the hypothesis that the endocrine part of the pancreas in cystic fibrosis is involved in a similar process to that affecting the exocrine cells

We found an overall diminished output of insulin in the 17 children studied 4 of them did have a normal output The glucagon response was variable The basal fasting glucagon level was higher in the children with cystic fibrosis and the response to arginine varied from very low to excess The most interesting group were four children who had a very high glucagon response following arginine and also had a high fasting glucagon level This was associated with low fasting blood sugar the glucose and insulin responses to arginine being very low It is possible that the high glucagon response could be secondary to hypoglycaemia (15) All children studied were fasted for a similar period and in view of the apparently normal glycogen stores and the normal response to glucagon in two of the children in this particular group we are unable to explain the hypoglycaemia

Porte et al (20) have shown low circulating insulin levels following epinephrine infusion High circulating catecholamines have been reported by Barbero & Braddock (1) in cystic fibrosis We considered this a possible reason for the insulinopenia However Handwerger et al (9) found that an infusion of phentolamine given two hours after a GTT caused no change in the insulin level Hence it is unlikely that a raised circulating catecholamines could account for the low insulin/glucagon ratio in these four children Raised glucagon levels have been found in certain states associated with increased metabolic rate such as following burns (27) and trauma (17) Two other conditions associated with high glucagon levels are liver cirrhosis (14) and renal failure (2) but there was no evidence to suggest the presence of either of these two conditions in the cystic fibrosis patients studied here

Analogous results have been described in chronic pancreatitis (12) in association with low insulin response

At the present time we cannot state with certainty the sources of the circulating glucagon which are measuring by radioimmunoassay Our pancreatic glucagon antiserum reacts with the C-Terminal portion of the glucagon molecule and this has been thought to be associated with a pancreatic glucagon specific antiserum This antiserum has low cross reactivity with extracts of gut but recent studies from our laboratory would suggest that there are some materials particularly in the stomach and colon that will cross react with this antiserum (7) In addition studies in dogs have suggested that the proximal gastrointestinal tract contains material very similar to pancreatic glucagon and indeed circulating pancreatic glucagon has been found to rise following pancreatectomy (16 25)

It is therefore possible that the glucagon levels which we are recording in this study especially in the insulinopenia patients are being derived from extra pancreatic sources Unless glucagon radioimmunoassays are modified and improved to recognize this the question will remain unanswered

We can conclude that insulin secretion is depleted in the majority of patients with cystic fibrosis However the conclusions with regard to glucagon secretion must remain guarded Some children do have diminished glucagon secretion but others have apparently

Acta Pædiatr Scand 66 205–209 1977

DIMINISHED LIMB BLOOD FLOW IN INFANTS WITH TRANSPOSITION OF THE GREAT VESSELS AN ADAPTATION TO CHRONIC HYPOXIA?

WILLIAM F POWERS and PAUL R SWYER

From th Research Institute of the Hospital for Sick Children and the Department of Pediatrics University of Toronto Toronto Canada

ABSTRACT Powers W F and Swyer P R (Research Institute of the Hospital for Sick Children and the Department of Pediatrics University of Toronto Toronto Canada) Diminished limb blood flow in infants with transposition of the great vessels An adaptation to chronic hypoxia Acta Paediatr Scand 66 205 1977.—Calf blood flow was measured by venous occlusion plethysmography using a mercury in rubber strain gauge in infants with transposition of the great vessels (TGV) and in comparable infants free from cardiopulmonary disease Resting calf blood flow in the infants with TGV was 3 6±0 8 ml/100 ml/min while in the control group flow was 6 8±2 3 ml/100 ml/min a highly significant difference We postulate that newborns with TGV decrease their resting calf flow in response to chronic hypoxia

KEY WORDS Peripheral circulation transposition of the great vessels hypoxia

Despite severe hypoxemia many infants with cyanotic congenital heart disease thrive Apparently tissue hypoxia and anaerobic metabolism are not the inevitable consequences of this severe hypoxemia To avoid hypoxia to critical tissues the infant could decrease his metabolic rate improve his oxygen extraction and/or alter his cardiac output and its distribution We have studied term infants with transposition of the great vessels (TGV) and found that their limb blood flow is lower than normal We report here our results and speculate that the human newborn at least in part copes with chronic hypoxemia by altering his peripheral circulation

MATERIALS AND METHODS

The clinical characteristics of the infants with TGV are given in Table I All were studied within the first week of life and all had undergone a balloon septostomy for [illegible] TGV None had had the Blalock Hanlon operation at the time of the study though four had this procedure later Some were digitalized at the time of study (Table I) but none had received diuretics for at least 24 hours prior to the time of study None were in clinically evident congestive cardiac failure at the time of the blood flow study though two may have been in early failure These two infants are described in Tables I and 3 but not included in statistical analyses All infants were resting quietly No anesthesia or sedatives were used Infants of comparable weight and postnatal age free from significant cardiorespiratory disease when studied were controls (Tables 2 and 4) None were receiving phototherapy at the time of study (20 24) After an explanation of the nature of these studies the parents gave verbal consent that they be carried out

The infants were studied in their incubators in a neutral thermal environment (16) at least two hours after the last feeding (25) Blood flow was measured exclusively in the left leg *for its vessels had* not been manipulated during the prior catheterization and septostomy procedure nor had they sustained arterial or venous punctures One cyanotic infant had had an umbilical arterial catheter in place which had been removed prior to the flow study Our previous work has shown that the prior use of an umbilical arterial catheter does not affect subsequent blood flow to the limb (21)

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Submitted May 17 1976
Accepted Oct 8 1976

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Table 1 *Some clinical characteristics of infants with TGV*

Initial	Description	Age (h)	Birthweight (g)	Resp rate (frequency)	Heart rate (beats/min)	Digitalis
T	Intact septum small PDA B H[b]	87	3 000	32	141	Yes
R	Intact septum	123	3 601	36	135	Yes
P	Intact septum B H[b]	77	3 390	40	128	Yes
K	Intact septum	142	2 640	32	144	No
R	Small VSD small PDA	70	3 460	40	157	Yes
L	Intact septum	84	3 125	32	110	No
S	Intact septum small PDA B H[b]	87	3 240	38	126	No
R	Intact septum B H[b]	120	3 317	48	126	Yes
		98 8±28	3 322±301	37 3±5 5	133 4±14	
E	With VSD	83	3 000	40	150	Yes
H[d]	Intact septum	74	3 400	58	129	No

Digitalized at time of study

[b] Needed Blalock Hanlon operation after initial septostomy and after blood flow study

Patient E went into overt failure three days after the peripheral circulatory studies Since he might have been in early failure at the time of the study his data are treated separately

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previously described (21 23) In the present study the *technique was modified in that the gauge was applied with* an initial tension of 10 g Also after initial calibration at room temperature (23–26 C) the gauge was placed in an environment which approximated limb temperature (33–35 C) and a new baseline was established at this raised environmental temperature We had previously established that the increased temperature while causing a *baseline shift did not affect the slope or span of calibration* Re-establishment of the baseline at or near skin temperature largely eliminates the need for temperature compensation (23)

At least six determinations of resting flow were made in rapid succession on each infant The mean was calculated and is expressed as a single value for resting flow in Ta*bles 3 and 4*

Blood samples were drawn at the time of blood flow *studies either by arterial stab or arterialized heel prick* and were analyzed for Pa_{O_2} pH Pa_{CO_2}[1] and hematocrit A previous study from our nursery had shown that pH and Pa_{CO_2} determined on heel prick samples are comparable to values determined on samples drawn through an umbilical arterial catheter over the range in question while heel prick Pa_{O_2} would if anything underestimate the true dif*ference in Pa_{O_2} in cyanotic and acyanotic infants* (15) Systolic blood pressure was measured with an ultrasonic flow detector (17) [2] Mean values for each parameter of the two groups were compared by Student s *t* test

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Table 2 *Some clinical characteristics of non cyanotic control infants*

Initial	Diagnosis/reason for referral	Age (h)	Birthweight (g)	Resp rate (frequency)	Heart rate (beats/min)
B	Transitional circulation (recovered)	101	3 740	44	118
M	?Aspiration	40	3 710	48	118
B	?Tracheo esophageal fistula	117	3 770	38	124
D	Hematemesis	110	? 800	40	128
M	ABO incompatibility	58	3 470	40	170
S	Transient tachypnea	67	3 120	40	150
S	Ambiguous genitalia	137	3 290	36	148
L	ABO incompatibility	108	3 200	36	13?
		92 3±33 4	3 388±347	40 3±4 0	129 8±13

Table 3 *Measurements of infants with TGV*

Initial	$\dot{Q}$ (ml/min/100 ml tissue)	Systolic blood pressure (mmHg)	Pa_{O_2} mmHg	Pa_{CO_2} mmHg	pH	Hct%	Sample site	F_IO_2
T	4 ?5	85	27	39	7 37	48	Art	0 21
R	3 24	75	29	30	7 37	48	Art	0 21
P	? 06	78	?6	34	7 40	55	Art	0 40
K	4 06	77	?6	31	7 39	45	Art	0 21
R	4 50	90	3?	36	7 40	50	Art	0 21
L	3 12	8?	27	38	7 32	58	Art	0 40
S	3 27	75	?1	30	7 47	54	Art	0 30
R	4 04	84	?4	38	7 46	44	Art	0 40
	3 60±0 80	80 8±5 4	?6 5±3 3	34 5±3 8	7 40±0 05	50 3±3 0		
E	4 83	82	28	33	7 56	51	Art	0 21
H	3 67	80	29	33	7 40	46	Art.	0 ?1

RESULTS

Calf blood flow (mean±S D) in our control infants was 6 8±2 3 ml/100 ml/min (Table 4) a value similar to those reported previously for well term infants (16 20 22 25) In contrast mean flow in the infants with TGV was 3 6±0 8 ml/100 ml/min (Table 3) This 48% decrease in flow was highly significant t=3 55 p<0 01 Average Pa_{O_2} in the infants with cyanotic heart disease was 26 5±3 3 mmHg significantly different from the mean Pa_{O_2} of 61 0±10 1 mmHg in the control infants t=8 63 p<0 01 Mean birth weight postnatal age pH Pa_{CO_2} pulse and respiratory rate systolic blood pressure and hematocrit were not significantly different in the two groups

DISCUSSION

Celander (7) found that normal newborn infants made acutely hypoxic decreased their peripheral flow He further demonstrated a more pronounced peripheral vasoconstriction in the asphyxiated human neonate (6)

Experimental physiologists have shown that the newborn goat (13) and the mature fetal lamb (1 4 5 8 12 14) similarly respond to acute hypoxemia by decreasing their femoral lower aortic or carcass blood flow while placental cardiac and carotid flow increases Cardiac output remains the same or decreases (4 8 10) Thus teleologically speaking in acute hypoxia the periphery is restricted in order to supply more vital organs so as to

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B	5 36	95	6?	37	7 4?	58	H P	0 21
M	6 16	70	67	37	7 43	54	H P	0 ?1
B	8 4	90	56	36	7 43	47	H P	0 ?1
D	5 57	85	55	39	7 40	43	H P	0 ?1
M	3 73	75	58	31	7 44	58	H P	0 21
S	8 65	80	55	?9	7 40	53	Art	0 30
S	11 0	76	5?	36	7 34	53	Art	0 21
L	6 01	86	83	?9	7 44	54	H P	0 21
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maximize the benefit from a compromised global oxygen supply This adjustment obtains despite constancy of Pa_{CO_2} (8, 11, 13) In the acutely asphyxiated experimental fetus where hypercarbia and acidemia are superimposed upon hypoxia peripheral flow is even further decreased (1, 8 11 18)

We believe that the diminished peripheral flow which we have documented in newborn infants with TGV represents their adaptation to chronic hypoxia and parallels the hypoxia induced circulatory changes seen acutely in newborn humans and experimental animals

The conditions under which our patients were studied do not explain the decrease in peripheral flow which we have observed for factors such as temperature (2 16) interval since feeding (25) phototherapy (20 24) age and birth weight (6 16) were carefully controlled The reduced flow in infants with TGV is not due to an increased hematocrit for the hematocrits of the two groups were comparable and differences in true hematocrit that might be attributed to sampling methods at this age are probably insufficient to explain viscosity changes large enough to account for the observed reduction in flow (3 19)

Since all the cyanotic infants we studied had only one lesion TGV it is possible that the observed flow reduction is explained by some circulatory peculiarity of TGV For instance it is theoretically possible that in TGV because of the impediment to mixing of the pulmonary and systemic circuits the systemic circulation is underfilled in comparison to the pulmonary circuit The frequent finding of pulmonary plethora in infants with TGV is consistent with this hypothesis Thus the systemic hypoperfusion we have measured would be explained not by hypoxia but by systemic hypovolemia We do not believe that this particular mechanisms was operating for the two infants who might have been in marginal failure at the time of study (Patients E and H in Table 1) would have been most likely to have had an overloaded pulmonary circuit and a depleted systemic circuit Hence their systemic peripheral flows should have been but indeed were not lower than the rest

Moreover Corpus & Hait (9) measured limb blood flow in older children with a different cyanotic lesion Tetralogy of Fallot and found that in these chronically hypoxic patients peripheral flow was also reduced Thus since acute hypoxia decreases lower body circulation in fetal and neonatal animals since the human neonate responds to acute hypoxia by peripheral vasoconstriction and since older children with other cyanotic heart lesions show diminished peripheral circulation we believe that chronic hypoxia is the most plausible explanation for the decrease in peripheral flow which we have demonstrated in newborns with TGV

ACKNOWLEDGEMENTS

This study was supported by a grant from the Ontario Heart Association We wish to thank Ms Antoinette Zitman R N and Mr Robert Adams for their assistance

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Submıtted Febr ? 1976
Accepted Sept 17 1976

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maximize the benefit from a compromised global oxygen supply This adjustment obtains despite constancy of Pa_{CO_2} (8 11 13) In the acutely asphyxiated experimental fetus, where hypercarbia and acidemia are superimposed upon hypoxia peripheral flow is even further decreased (1 8, 11 18)

We believe that the diminished peripheral flow which we have documented in newborn infants with TGV represents their adaptation to chronic hypoxia and parallels the hypoxia induced circulatory changes seen acutely in newborn humans and experimental animals

The conditions under which our patients were studied do not explain the decrease in peripheral flow which we have observed for factors such as temperature (2 16) interval since feeding (25) phototherapy (20 24) age and birth weight (6 16) were carefully controlled The reduced flow in infants with TGV is not due to an increased hematocrit for the hematocrits of the two groups were comparable and differences in true hematocrit that might be attributed to sampling methods at this age are probably insufficient to explain viscosity changes large enough to account for the observed reduction in flow (3 19)

Since all the cyanotic infants we studied had only one lesion TGV it is possible that the observed flow reduction is explained by some circulatory peculiarity of TGV For instance it is theoretically possible that in TGV because of the impediment to mixing of the pulmonary and systemic circuits the systemic circulation is underfilled in comparison to the pulmonary circuit The frequent finding of pulmonary plethora in infants with TGV is consistent with this hypothesis Thus the systemic hypoperfusion we have measured would be explained not by hypoxia but by systemic hypovolemia We do not believe that this particular mechanisms was operating for the two infants who might have been in marginal failure at the time of study (Patients E and H in Table 1) would have been most likely to have had an overloaded pulmonary circuit and a depleted systemic circuit Hence their systemic peripheral flows should have been but indeed were not lower than the rest

Moreover Corpus & Hait (9) measured limb blood flow in older children with a different cyanotic lesion Tetralogy of Fallot and found that in these chronically hypoxic patients peripheral flow was also reduced Thus since acute hypoxia decreases lower body circulation in fetal and neonatal animals since the human neonate responds to acute hypoxia by peripheral vasoconstriction and since older children with other cyanotic heart lesions show diminished peripheral circulation we *believe that chronic hypoxia is the most plausible* explanation for the decrease in peripheral flow which we have demonstrated in newborns with TGV

ACKNOWLEDGEMENTS

This study was supported by a grant from the Ontario Heart Association We wish to thank Ms Antoinette Zitman R N and Mr Robert Adams for their assistance

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Acta Pædiatr Scand 66 211–217 1977

TRANSFER FACTOR IN CHRONIC AND RECURRENT RESPIRATORY TRACT INFECTIONS IN CHILDREN

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From the Institute of Biomedical Sciences School of Medicine University of Tampere and from the Children s Department Tampere Central Hospital Tampere Finland

ABSTRACT Grohn P (The Institute of Biomedical Sciences School of Medicine University of Tampere and the Children s Department Tampere Central Hospital Tampere Finland) Transfer factor in chronic and recurrent respiratory tract infections in children Acta Paediatr Scand 66 211 1977.—Five cases with abnormal sensitivity to respiratory tract in fections are described The cases showed a marked impairment in their cell mediated im munity state Administration of a chromatographically purified transfer factor component increased the skin test sensitivity to common recall antigens Interestingly a similar effect in skin reactivity was observed with repeated skin tests alone when antigen concentrations initially high enough to cause a positive reaction were used Neither the administration of transfer factor nor skin testing with high antigen concentrations had an effect on blast trans formation percentages The therapy with chromatographically purified transfer factor appeared promising on the clinical condition of the patients

KEY WORDS Transfer factor cellular immunity respiratory infections

Chronic or recurrent respiratory infections including episodes of tonsillitis otitis sinus itis bronchitis and pneumonitis are frequent ly seen in pediatric patients Occasionally specific causes such as defects in the granulo cyte function or in humoral immune response can be demonstrated but only in a minority of the whole patient group In some patients a specific or a nonspecific disturbance in the cellular immunity may be responsible for the impaired resistance to infection

Human dialyzable transfer factor (dTF) described by Lawrence was claimed to induce delayed hypersensitivity to recipients having a negative skin reactivity before the transfer (8) Despite the lack of clear knowledge of the biological mechanism of TF activity dTF has successfully been used as a therapeutic agent in a variety of conditions with decreased re sistance to infections (3)

Our group has fractionated dTF by gel filtra tion chromatography with Sephadex G 10 and found that the major activity was present in a fraction designed VIa and eluting character istically late from the column This chromato graphically purified TF was subsequently used in successful therapeutic trials in cases of juvenile rheumatoid arthritis miliary lupus of the face and in chronic urinary tract infections (4 5 1)

The present work deals with the state of cellular immune response in a group of chil dren suffering from repeated respiratory infec tions without a demonstrable defect in their antibody synthesis and with normal granulo cyte function tests In most of these patients skin testing revealed a decreased delayed hypersensitivity to common recall antigens such as tuberculin and oidiomycin and thera peutic trial with fraction VIa from dTF had a beneficial effect

MATERIALS AND METHODS

Description of the patients

Five children with an increased sensitivity to re piratory tract infections were included in this report (Table I)

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Chronic or recurrent respiratory infections including episodes of tonsillitis otitis sinusitis bronchitis and pneumonitis are frequently seen in pediatric patients Occasionally specific causes such as defects in the granulocyte function or in humoral immune response can be demonstrated but only in a minority of the whole patient group In some patients a specific or a nonspecific disturbance in the cellular immunity may be responsible for the impaired resistance to infection

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MATERIALS AND METHODS

Description of the patients

Five children with an increased sensitivity to respiratory tract infections were included in this report (Table 1)

Table 1 *Description of the patients and transfer factor therapy*

Patient	Sex	Age (y)	Diagnosis	Number of injections	Follow up time (mo)
1	F	6	Bronchitis chr *Prurigo Besnier* *Alopecia totalis*	9	19
2	M	12	Bronchitis chr tonsillitis residivans	4	17
3	M	4	Bronchitis chr *Otitis residivans* Atrophia cerebri cum epilepsiam	15	19
4	M	2	Bronchitis residivans Candidiasis unguis	8	18
5	M	2	Bronchitis residivans Tonsillitis residivans	10	18

Investigations which were performed in order to find a possible cause for their sensitivity to infections revealed *normal levels in lymphocyte and granulocyte counts* as well as normal granulocyte function evaluated with the NBT test and normal levels of immunoglobulins and complement components C1 C3 and C4 in each case T and B cell levels and tests to evaluate the phagocytosis function of blood monocytes had not been performed

Controls

The skin test sensitivity in healthy children was estimated in 15 cases and the blast responses to PHA PPD and OM in 7 cases These results have been reported earlier (4) The booster effect of repeated skin testing on skin reactivity was evaluated in 5 cases of juvenile rheumatoid arthritis (JRA) 3 cases with recurrent respiratory tract infections (RI) and 9 cases with urinary tract infections (UTI) Two control groups (A and B) were formed Initially the highest concentration of PPD needed for a positive skin reaction was estimated in both groups similarly to the patient group Patients in group A received PPD at the highest concentration which still gave a negative reaction in the initial test while the patients in group B also received the concentration which gave a positive reaction initially (Table 2)

Purification of dTF

Human leucocyte dialyzates derived from pooled buffy coat cells obtained from healthy blood donors (The Finnish Red Cross Transfusion Service Helsinki Finland) were fractionated on a Sephadex G 10 column as previously described (6) Fraction VIa eluting after the total volume of the column

$$K_d = \frac{V - V}{V_t - V} = 1\ 68$$

was pooled lyophilized and weighed

It was redissolved in physiological saline at a concentration of 15µg/ml and sterilized with Millipore filtration Aliquots of 1 0 ml volume were stored at −20 C for further use

Skin testing

Skin testing was performed by using tuberculin (PPD State Bacteriological Laboratory Copenhagen Denmark) and oidiomycin (OM) Dermatophytin O Hollister Stier Spokane WA USA) PPD was used in concentrations of 0 1 TU 1 TU 10 TU and 100 TU and OM in dilutions of 1 500 and 1 50 The skin testing was started with weak concentrations and repeated at one week intervals until either a positive reaction was noticed or the highest concentration of the test antigen was used The test antigen was injected at a dose of 0 1 ml on volar sites of upper arms and the results were registered after 24 48 and 72 hours An erythema and induration of more than 5×5 mm in diameter was regarded as a positive reaction Grading of skin tests in presented in Table 2

Lymphocyte stimulation

Phytohemagglutinin (PHA Phytohemagglutinin P Difco Laboratories Detroit Mich USA) PPD and OM were used as antigens The blast transformation test was performed by a method described earlier (6) Leucocytes 2×10[6] separated by Ficoll Isopaque gradient were cultured in plastic dishes in RPMI 1640 medium (Orion Helsinki Finland) containing 20% Fetal Calf Serum (Flow Laboratories Irvine Scotland) Cell were harvested after three days (PHA) and six days (PPD OM) and cell preparations were made by Shandon cytocentrifuge for May Grunwald-Giemsa staining The percentage of blasts from all lymphoid cells was estimated with the aid of an ocular grid

General plan of the study

Before the administration of fraction VIa the in vivo reactivity to PPD and OM was evaluated by skin testing and the in vitro reactivity to these antigen and PHA by the blast transformation method Together with the first

Table 2 *The effect of repeated negative (control group A) and positive (control group B) skin testing with tuberculin on skin test sensitivity in patients suffering from juvenile reumatoid arthritis recurrent respiratory infections and urinary tract infections*

Grading of the skin tests PPD 100TU−=0 100 TU+=1 10TU+=2 1TU+=3 0 1TU+=4 OM 1 50−=0 1 50+=1 1 500+=2 JRA=juvenile rheumatoid arthritis RI=respiratory infection UTI=urinary tract infection LUTI=lower urinary tract infection RPN=recurrent pyelonephritis

Patient	Diagnosis	First test	Grade	Third test	Grade
Control group A					
1	JRA	100TU+	1	10TU−	1
	JRA	100TU−	0	100TU−	0
3	JRA	10TU+	?	1TU−	2
4	JRA	100TU+	1	10TU−	1
5	JRA	10TU+	2	1TU+	3
6	RI	100TU+	1	10TU−	1
7	RI	100TU+	1	10TU−	1
8	RI	10TU+	?	1TU−	?
9	UTI	10TU+	?	1TU−	2
10	UTI	10TU+	2	1TU−	2
11	UTI	1TU+	3	0 1TU−	3
Mean ±S D			1 5±0 8		1 6±0 9
Control group B					
1	LUTI	10TU+	?	1TU−	2
?	RPN	100TU+	1	10TU+	?
3	RPN	1TU+	3	0 1TU+	4
4	RPN	10TU+	2	1TU+	3
5	RPN	100TU+	1	10TU+	2
6	RPN	10TU+		1TU+	3
Mean ±S D			1 8±0 8		? 7±0 8

injection of fraction VIa the skin testing was repeated with the concentrations which had given negative responses initially The effect of fraction VIa on lymphocyte stimulation was registered for a week later Occasionally both the in vivo and in vitro tests were repeated during the trial Injections of fraction VIa were given at the beginning of the trial once a week two times and thereafter once or twice a month for 5 to 1? months The evaluation of the therapy was done by registering the infectious episodes and the need of antimicrobial therapy during and after the trial

RESULTS

The effect of fraction VIa on skin testing

Before the administration of fraction VIa cases 1 and 2 showed skin test anergy to PPD while patients 3 and 4 were skin test positive to 100 TU and case 5 to 10 TU of PPD Testing with OM showed that only case 2 had a positive reaction After the injection of fraction VIa the skin test reactions were markedly strengthened in all cases with PPD and three cases with OM (Table 3)

The effect of fraction VIa on lymphocyte stimulation

Blastogenic responses to PPD were of medium strength in cases 3 4 and 5 Generally administration of fraction VIa did not alter the patients in vitro reactivity in lymphocyte stimulation tests Responses to PHA were within normal limits before and after TF therapy and threre was equally no significant change in the responses to PPD or OM in spite of a clear increase in skin reactivity to the same antigens (Table 3)

Control groups

In healthy children the mean skin test sensitivity to PPD was between 1 and 10 TU and to OM between dilutions 1 500 and 1 50 (Table 3 Fig 1) The mean blast transformation responses were 82 1 to PHA 3 2% to PPD and 1 4% to OM (Table 3) Repetition of skin tests caused a significant increase in con

Table 3. *Skin reactivity to PPD and OM and the blast transformation responses to PPD, OM and PHA before and after the administration of fraction VIa*

Grading of the skin tests: PPD 100 TU − = 0, 100 TU + = 1, 10 TU + = 2, 1 TU + = 3, 0.1 TU + = 4. OM 1:50 − = 0, 1:50 + = 1, 1:500 + = 2. p_1 = degree of significance between the results before and after the administration of fraction VIa. p_2 = degree of significance between the results of patients and controls. ns = not significant

	Skin testing				Lymphocyte stimulation					
	PPD responses		*OM responses*		*PPD responses (blast %)*		*OM responses (blast %)*		*PHA responses (blast %)*	
Patient	Before	After	Before	After	Before	After	Before	After	Before	After
1	0	1	0	1	0.0	0.0	0.0	0.4	86.0	57.2
2	0	1	1	2	0.2	0.2	0.2	0.2	56.6	60.0
3	1	3	0	0	7.2	0.7	0.0	0.0	83.4	68.6
4	1	3	0	1	4.0	3.6	0.0	0.0	52.8	69.0
5	2	3	0	0	3.0	3.0	0.0	0.0	80.2	72.6
Mean ±S.D.	0.8±0.8	2.2±1.1	0.2±0.4	0.8±0.8	2.9±3.0	*1.6±1.7*	0.0	*0.1±0.2*	*71.8±15.8*	*65.5±6.5*
p_1		≤0.0025		≤0.05		ns		ns		ns
Controls	2.1±0.4		0.7±0.5		3.2±3.0		1.4±1.2		82.1±12.2	
p_2	≤0.0025	ns	≤0.025	ns	ns	ns	≤0.005	≤0.005	≤0.025	≤0.005

trol group B, where is only one case in control group A had an increased reactivity after the repeated skin testing (Table 2.)

The clinical effects of fraction VIa

Case 1, a girl, suffered from recurrent bronchitis which began a few months after her birth in November 1968. At the age of three years she had an eruption of an atopic exzema which she has had ever since. Simultaneously she lost her hair as well as eyelashes and eyebrows. The exzema was not treated topically or systemically. Her living conditions were standard before, during and after the trial. She received 9 injections of fraction VIa during a 10 month period, starting in September 1974. During this time she had only one episode of infection and during the nine months follow-up period, since the discontinuation of the injections, she had only one attack of otitis, not requiring antibiotics. Her eyelashes, eyebrows and hair have started to grow, and she no longer needs to wear a wig. The treatment had no effect on the atopic exzema (Table 1).

Case 2, a boy, had had chronic bronchitis beginning a month after his birth in June 1962. In addition, he had recurrent tonsillitis and during the last two years before the start of TF trial, he needed antibiotics either for bronchitis or tonsillitis on the average of once every other month. He received 4 injections of fraction VIa during six months, starting in November 1974. During that period and also the subsequent year, he has not had a single infection requiring antibiotics, but has only encountered influenza A during the epidemic in February 1976 (Table 1).

Case 3, a boy, had had repeated upper respiratory tract infections starting a few month after his birth in October 1970. In 1972 a biopsy from the bronchial mucous membrane showed changes indicating chronic bronchitis. He has had epileptic attacks from the age of two years and a pneumoencephalogram showed cerebral atrophy. The therapeutical trial with fraction VIa was started in September 1974 and he received 12 injections during 13 months. During that time he had three episodes of bronchitis, which was considerably less than before the therapy. However, four months after the discontinuation of the therapy he developed otitis and at this time his skin tests to 100 TU of PPD and 1:50

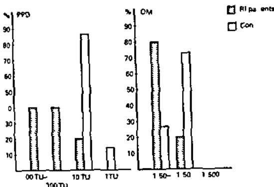


Fig 1 Percentile distribution of PPD and OM skin test values in control material and chronic or recurrent respiratory tract infections (*RI*) of children

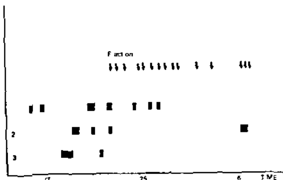


Fig 2 Case 3 Clinical history and the effect of transfer factor therapy (1=bronchitis 2=otitis 3=pneumonia)

dilution of OM were negative A new therapeutical trial has now been started with this case (Table 1 Fig 2)

Case 4 a boy had had recurrent bronchitis beginning a few months after his birth in March 1973 He had candidiasis in his nails since birth and a mild atopic exzema He had frequent otitis and on one occasion this was combined with Bell s paresis He received 8 injections of fraction VIa from October 1974 to July 1975 Since the beginning of this therapy he has had no respiratory infections and the candida has disappeared During the subsequent nine month period after therapy he has been free from symptoms However he did develop a lymphadenitis in his groin after a vaccination against polio (Table 1)

Case 5 a boy was born in September 1972 Since birth he had had attacks of bronchitis almost monthly In addition he had chronic laryngitis with constant *hoarseness* He received 10 injections of fraction VIa from October 1974 to September 1975 There were no respiratory infections during this period but on one occasion he had tonsillitis After the discontinuation of the therapy he had one episode of a common cold (Table 1 Fig 3)

DISCUSSION

The five cases selected to this preliminary trial on the effect of transfer factor on recurrent or chronic respiratory tract infections form a group with an exceptionally grave clinical history They all had recurrent episodes of infections beginning a few months after birth In four of the cases there was only one infectious episode requiring antibiotics during the trial or during the subsequent 9–12 months follow up period In view of the frequency of these episodes before the trial the effect of transfer factor therapy in these cases must be evaluated as promising The living conditions of the patients were considered constant before during and after the therapy as well as these periods included all the seasons of the year

The mechanism of action of TF in these cases as well as in general is however unknown TF was originally described as an informational molecule capable in transferring specific immunological information and it has



Fig 3 Case 5 Clinical history and the effect of transfer factor therapy (1=laryngitis 2=bronchitis 3=tonsillitis)

suggested to be composed of a polynucleotide polypeptide structure (8) Subsequent studies have shown that leucocyte dialyzates contain several functional substances and it is probable that some if not all of the transfer factor s activity is based on a nonspecific stimulatory mechanism (2) In the present study a highly purified TF preparation was used in which the presence of nucleotides or peptides has not been demonstrated Chemical studies on the composition of fraction VIa have revealed that its major component is uracil (7) In addition fraction VIa contains several unidentified small heterocyclic aromatic compounds (6)

Although the defect leading to impaired resistance to infections in these five cases is not known they all seemed to have a decreased cellular immune reactivity as defined by skin testing to recall antigens such as PPD and OM They do not demonstrate defects in humoral immunity and their complement levels were normal Also there was no defect in the granulocyte level or function A positive skin test to a recall antigen is a result of two events a specific one requiring functionally capable sensitized T cells and a nonspecific one expressed by monocytes and macrophages and mediated by lymphokines secreted by the stimulated T cells The fact that the in vitro tests for lymphocyte function were normal in some of the patients in spite of a weak skin reaction to PPD and that there was no alteration in the blastogenic responses of lymphocytes after the administration of fraction VIa even when the skin reactivity increased significantly would indicate that the responding cells in these patients were monocytes rather than lymphocytes It has been previously suggested that the action of TF is based on its effect on monocytes and the present results are in accordance to that view

It is of interest to note that repetition of skin testing caused a strengthening in skin test reactivity when skin testing was repeated in some patients with an antigen dose high enough to cause a positive skin reaction initially After repeated positive skin reactions the patients were observed to react positively to a lower antigen dose It is still unclear whether this observation is based simply on an immunogenic effect of the test antigen or whether the liberation of biologically active substances from the lymphocytes at the site of skin reaction might have more general effects and stimulate the whole monocyte population of the body Repetition of skin tests can either decrease or increase skin test sensitivity This has been shown to depend on antigen dose site of repetition interval of repetition and number of antigen doses (9) The way with which skin testing has been performed in this study excludes the possibility of the repeated skin tests to affect skin reactivity

ACKNOWLEDGEMENTS

This study has been supported by grants from The Sigrid Jusélius Foundation The Emil Aaltonen Foundation and from The State Medical Council of Finland

The generosity of The Finnish Red Cross Transfusion Service in providing us with buffy coat cells is greatly appreciated

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Submitted April 20 1976
Accepted Sept 27 1976

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Acta Pædiatr Scand 66 219–224 1977

TRANSFER FACTOR AND CELLULAR IMMUNE RESPONSE IN URINARY TRACT INFECTIONS IN CHILDREN

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ABSTRACT Anttila R Grohn P and Krohn K (The Institute of Biomedical Sciences School of Medicine University of Tampere and the Children s Department Tampere Central Hospital Tampere Finland) Transfer factor and cellular immune response in urinary tract infections in children Acta Paediatr Scand 66 219 1977 —Cellular immune responses in vivo and in vitro were studied in 20 children with chronic or relapsing urinary tract infections Skin tests revealed decreased immune responses to PPD in cases with chronic or recurrent pyelonephritis and to OM in these cases and in cases of lower urinary tract infections Blast transformation responses to PPD OM and PHA were at least as high as in controls Administration of chromatographically purified fraction from human leucocyte transfer factor resulted in a positive skin reaction with antigen concentration which before TF administration had caused a negative reaction The results suggest that the action of the transfer factor component used in this study is based on an immunologically nonspecific stimulation of the cellular immune response

KEY WORDS Transfer factor cellular immunity urinary tract infections

Urinary tract infections (UTI) form a major problem in pediatric practice partly because of their frequency (10) but especially because of their tendency to relapse (2) Several reasons for a susceptibility to recurrent or chronic UTI have been suggested but the reflux phenomenon and anatomical factors such as obstructive malformations or duplications are generally believed to be of greatest importance However other factors may operate in this condition as UTI are often seen in patients without any demonstrable anatomical defects In female patients the tendency to UTI often seems to be familial Also patients with minor or even gross malformation in the urinary tract do not always have a greater tendency for UTI

Immunological defects in recurrent or chronic UTI patients have been looked for but on most occasions they have normal serum immunoglobulin and complement levels as well as granulocyte function test and count in the peripheral blood (7)

The present study was undertaken to find out whether children with different forms of UTI would show impairment in their cellular immune reactivity Secondly we wanted to test the effect of dialyzable transfer factor (dTF) on the immune response We have previously purified a component from dTF and shown that it had promising therapeutic effect in cases with recurrent respiratory infections juvenile rheumatoid arthritis and miliary lupus of the face (3 4 5)

The present study shows that some of the patients with UTI have a decreased cellular immune reactivity and that this reactivity can be increased with chromatographically purified dTF (dTFc)

MATERIALS AND METHODS

Selection of the patients

The material consists of 0 children (1 boy 19 girls) aged from 7 to 14 years with the mean age being 8 1 years All of the patients had suffered from recurrent or chronic urinary tract infections but none of them had an acute episode of the UTI or any other infection at the



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Table 1 *Description of the patients*
IVP=intravenous pyelography MCG=mictiocystography

	Age	Sex	X ray findings IVP	MCG
CPN patients				
1	7	F	Local cortical atrophy	Normal
2	9	F	Parenchymal reduction	Reflux
3	6	M	Local cortical atrophy	Normal
4	5	F	Local cortical atrophy	Reflux
5	12	F	Parenchymal reduction *parenchymal* changes	Normal reflux *operated*
RPN patients				
6	7	F	Normal	Normal
7	13	F	Normal	Normal
8	3	F	Normal	Reflux neurogenic bladder
9	9	F	Normal	Normal
10	9	F	Normal	Normal
11	14	F	Normal	Normal
LUTI patients				
12	4	F	Double pelvis and ureter	Normal
13	2	F	Normal	Normal
14	10	F	Normal	Normal
15	7	F	Normal	Normal
16	14	F	Normal	Normal
17	9	F	Normal	Normal
18	9	F	Normal	Trabeculation of bladder
19	6	F	Normal	Normal
20	7	F	Normal	Normal

time of examination The patients were divided in three groups according to the following criteria

Group I consisted of 5 patients with chronic pyelonephritis (CPN) These patients had had episodes of acute pyelonephritis and they had changes in intravenous pyelography (IVP) and decreased renal function or pathological renal biopsy None of the patients were uremic

Group II had 6 patients with recurrent episodes of acute pyelonephritis (RPN) The diagnosis of acute pyelonephritis (APN) was based on fever micturition disturbances increased erythrocyte sedimentation rate (ESR) positive CRP positive blood culture and increased antibody titer to E coli antigen The urinary findings included proteinuria haematuria pyuria and a bacterial count of more than 10^5/ml in three voided urine specimens or in one urine sample taken with catheter or bladder puncture Transient decreased concentration capacity was also seen in the acute phase

Group III consisted of 11 patients with recurrent lower urinary tract infections (LUTI) These patients had pyuria They had a bacterial count of more than 10^5/ml in three voided urine specimens or in one urine sample taken with catheter or bladder puncture They had disturbances in micturition but neither fever increased ESR CRP nor other findings used for the diagnosis of acute pyelonephritis A description of the patients is given in Table 1

Controls

The in vivo reactivity to PPD and OM was tested in 15 healthy children (mean age 6 2 years) and the in vitro reactivity to PHA PPD and OM in 7 healthy children (mean age 6 0 years) The results have been described earlier (4)

Preparation of the chromatographically purified TF component

Leucocytes from buffy coats (from The Finnish Red Cross Transfusion Service Helsinki Finland) were used as a source of dTF The preparation of dTF has been described earlier (8) Shortly dTF was fractionated on a Sephadex G 10 column by using distilled water as eluent and the active fraction VIa eluting in the outer volume of the columns was collected This was freeze-dried diluted in physiological saline filtered through a 0 22 Millipore filter and used in subcutaneous doses of 15 μg/ml for systemic transfer

Skin testing

Skin tests were performed at weekly intervals with tuberculin (PPD State Bacteriological Laboratory Copenhagen Denmark) in concentrations of 0 1 TU 1 TU 10 TU and 100 TU of PPD and with oidiomycin (OM Dermatophytin O Hollister Stier Laboratories Spokane Wa USA) in dilutions of 1 500 and 1 50 On the first occasion the two weakest concentrations of PPD and OM were used In cases with negative PPD reactions 10 TU of PPD was given for a week later followed by *100 TU in those cases which were negative to 10 TU* The results were registered after 24 48 and 72 hours *An erythema and induration of more than* 5×5 mm in diameter was regarded as a positive reaction Grading of the skin test results was as follows PPD 0 1 TU+=4 1 TU+=3 10 TU+=2 100 TU+=1 and 100 TU−=0 OM 1 500+=2 1 50+=1 and 1 50−=0 Statistical evaluation was performed by using Student s *t* test

Lymphocyte stimulation

Cell cultures were made according to the method described earlier (8) 20 ml of venous blood was drawn into a heparinized plastic syringe Leucocytes were *separated by Ficoll Isopaque gradient and* 2×10⁶ cells were cultured in plastic dishes with RPMI 1640 (Orion Helsinki Finland) and 20% Foetal Calf Serum (Flow Laboratories Irvine Scotland) as the medium Phytohemagglutinin (PHA Phytohemagglutinin P Difco Laboratories Detroit Mich USA) PPD and OM were used as antigens Harvesting was made after three days (PHA) and six days (PPD OM) Cell preparations were made by Shandon cytocentrifuge for May Grunwald Giemsa staining The percentage of *blasts from all* lymphoid cells was counted with the aid of an ocular

Table 2 *Skin reactivity to PPD and OM before and after the administration of dTFc*

Grading of the skin tests PPD 100 TU − = 0 100 TU + = 1 10 TU + = 2 1 TU + = 3 0 1 TU + = 4 OM 1 50 − = 0 1 50 + = 1 1 500 + = 2

Patient	Skin testing PPD responses Before	After	OM responses Before	After
Chronic pyelonephritis				
1	0	2	0	0
2	0	1	0	1
3	1	3	0	1
4	2	3	1	1
5	2	4	0	1
Mean ±S D	1 0±1 0	2 6±1 1	0 2±0 4	0 8±0 4
p		≤0 0005		≤0 05
p_1	≤0 0005	ns	≤0 025	ns
Recurrent acute pyelonephritis				
6	0	2	0	0
7	1	2	0	1
8	1	3	1	1
9	2	3	1	1
10	2		0	
11	3	3	1	1
Mean ±S D	1 7 ±1 3	2 8±0 8	0 5±0 5	0 8 ±0 4
p		≤0 0125		ns
p_1	ns	ns	ns	ns
Lower urinary tract infections				
12	0	2	0	0
13	1		0	0
14	2	2	0	0
15		3	0	0
Mean ±S D	1 2 ±1 2	2 2 ±0 5	0 0 ±0 0	0 0 ±0 0
p		0 05		ns
16	2		1	
17	2		1	
18	3		0	
19	3		0	
20	3		0	
Mean ±S D	2 0 ±1 0	2 ±0 5	0 2 ±0 4	0 0 ±0 0
p	ns	ns	≤0 001	≤0 0001
Controls	2 1 ±0 4		0 7 ±0 5	
Total material	1 6 ±1 0	5 ±0 8	0 5 ±0 3	0 6 ±0 5
p		≤0 0001		ns
p_1	0 05	ns	ns	ns

p = degree of significance between the results before and after the administration of dTFc
p_1 = degree of significance between the results of patients and controls
ns = not significant

grad The percentages in cultures without antigen were subtracted from the percentages in cultures with antigen

Course of the study

On the first occasion the lymphocyte cultures were taken and the skin testing was started One week after the last skin tests were made the effect of dTFc was estimated by injecting a single dose of 15 µg of dTFc subcutaneously and by repeating the highest skin test concentration which gave a negative response initially The lymphocyte cultures were repeated a week later

RESULTS

The state of cellular immunity of the patients

Decreased blast transformation responses to PHA were seen only in cases 2 and 7 The mean blast percentages of PHA differed significantly from the control material but were in normal ranges The blast percentages of PPD and OM showed no significant differences to the control material (Table 3)

The mean skin test value to PPD was 1 0 in group I 1 7 in group II and 2 0 in group III (for grading see methods) The mean value for all the groups was 1 6 Reactivity to PPD differed significantly from the control material in patient group I The responses to OM were 0 2 0 5 0 2 and 0 3 in groups I II III and in all of the groups respectively A significant difference to controls was seen in patient groups I III and the total test groups (Table 2) The percentile distribution of the skin test results of the patients and the controls is presented in Fig 1

The effect of dTFc

Blast transformation After the administration of dTFc an increase in blast percentages was

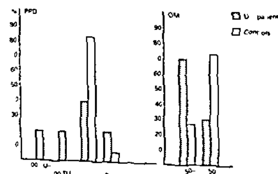


Fig 1 Percentile distribution of PPD and OM skin test values in childrens urinary tract infections and controls

Table 3 *Blast transformation responses to PPD OM and PHA before and after the administration of dTFc*

Patient	Lymphocyte stimulation: PPD responses (blast %)		OM responses (blast %)		PHA responses (blast %)	
	Before	After	Before	After	Before	After
Chronic pyelonephritis						
1	0 0	0 0	0 0	0 0	54 4	59 3
2	0 4	2 2	0 0	0 0	37 6	39 0
3	0 5	1 3	0 2	0 7	82 5	82 8
4	4 8	7 4	3 8	4 0	66 0	50 8
5	33 1	21 6	0 7	3 8	75 0	85 1
Mean ±S D	7 8±14 3	6 7±8 9	0 9±1 6	1 8±1 9	63 1±17 7	63 4±20 1
p_1		ns		ns		ns
p_2	ns	ns	ns	ns	≤0 005	≤0 005
Recurrent acute pyelonephritis						
6	0 2	9 2	0 4	4 7	67 0	71 0
7	1 2	0 2	0 0	0 0	47 6	39 4
8	21 0	25 4	17 9	6 5	83 4	76 6
9	2 2	1 8	1 4	1 2	65 4	54 4
10	8 7		0 4		77 6	
11	0 6	0 2	0 6	0 0	52 3	62 6
Mean±S D	5 7±8 2	7 3±10 8	3 6±6 8	2 5±3 0	66 7±12 9	60 8±14 6
p_1		ns		ns		ns
p_2	ns	ns	ns	ns	≤0 025	≤0 01
Lower urinary tract infections						
12	4 2	2 4	0 0	0 1	64 2	84 1
13	6 6	8 0	5 6	8 2	65 8	63 6
14	9 2	6 0	0 0	0 0	64 0	52 4
15	2 8	1 2	0 0	0 0	59 4	67 0
Mean ±S D	5 7±5 0	4 4±3 1	1 4±2 0	2 1±4 1	63 3±6 8	65 5±13 3
p_1		ns		ns		ns
16	14 4		2 0		80 0	
17	8 0		0 0		62 0	
18	12 2		0 6		63 8	
19	30 8		0 4		77 8	
20	5 0		0 2		65 4	
Mean ±S D	10 3±8 5	4 4±3 1	0 9±2 0	2 1±4 1	64 7±7 1	65 5±13 3
p_2	≤0 025	ns	ns	ns	≤0 0025	≤0 05
Controls	3 2±3 0		1 4±1 2		82 1±12 2	
Total material	8 3±10 0	6 2±8 0	1 7±4 0	2 1±2 8	65 6±11 8	63 1±16 3
p_1		ns		ns		ns
p_2	≤0 05	≤0 05	ns	ns	≤0 0025	≤0 0025

p_1=degree of significance between the results before and after the administration of dTFc
p_2=degree of significance between the results of patients and controls

observed in some individual cases This was seen especially with PPD in the most anergic patients in group I However in the whole material or even in the different groups as a whole the blast percentages to PPD and OM before and after the administration of dTFc did not significantly differ (Table 3)

Skin testing Administration of dTFc caused a clear strengthening of the skin test responses The mean skin test values after the transfer (2 5 to PPD and 0 5 to OM) differed significantly from the values before the transfer but did not differ from the values of the control group

DISCUSSION

Cellular immune reactivity in children suffering from different forms of urinary tract infections was studied by skin testing and by in vitro blast transformation tests The patients series was divided into three groups according to severity of the disease Impaired reactivity by skin testing was observed in all three groups particularly in groups I and II with chronic or relapsing pyelonephritis This observation was especially clear with PPD as the antigen while the candida antigen oidiomycin did not clearly differentiate the patient group from the controls As all of the patients in the present series have been vaccinated with BCG we feel that PPD is an especially suitable antigen In controls significant differences were not noticed in skin test values to PPD or OM in various age groups Contrary to the skin testing the blast transformation tests were not abnormal in the patients Only two patients had a weak PHA response and the blast percentages with PPD were in fact higher with patients than with controls A positive skin reaction requires both the antigen specific reactivity of the target cells such as blood monocytes to the mediators secreted by the stimulated lymphocytes Thus skin testing covers a wider range of reactivities than the in vitro blast transformation test

A chromatographically purified fraction of human dialyzable transfer factor dTFc was used in this study to correct the impaired cellular immune reactivity The results were similar to those observed earlier by us in patients with generalized sarcoidosis juvenile rheumatoid arthritis and in chronic or recurrent respiratory tract infections in children One injection of dTFc virtually normalized the skin reactivity to PPD whereas no alteration was seen in the blast transformation test This kind of dissociation in cell mediated immune response after transfer factor therapy has been reported previously by many authors (1 6 9)

Our results suggest that the failure in cellular immune reactivity in the patients is not in the capacity of lymphocytes to recognize and respond to the antigen but either in the lymphokine secretion or in the reactivity of target cells to the lymphokines A similar situation has previously been observed with uremic patients but none of the cases in the present series had uremia Also none of the patients had signs of acute infection and the drugs received by the patients are not known to cause immunosuppression One may therefore conclude that the observed decreased cellular immune reactivity was not the result of the disease and could thus be of etiological significance for the process

The present results suggest that some of the children with chronic or relapsing urinary tract infections do have an impaired cellular immune reactivity and that this can be corrected with transfer factor Whether this observation has any therapeutical implications is currently under investigation

ACKNOWLEDGEMENTS

This study has been supported by grants from The Sigrid Jusélius Foundation The Emil Aaltonen Foundation and from the State Medical Council of Finland

The generosity of The Finnish Red Cross Transfusion Service in providing us with buffy coat cells is greatly appreciated

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Submitted April 20 1976
Accepted Sept 27 1976

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Acta Pædiatr Scand 66 225–227 1977

NEONATAL SEPTIC OSTEO ARTHRITIS DUE TO UMBILICAL ARTERY CATHETERISATION

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ABSTRACT Knudsen F U and Petersen S (Department of Paediatrics Glostrup Hospital Glostrup Denmark) Neonatal septic osteo-arthritis due to umbilical artery catheterisation Acta Paediatr Scand 66 225 1977.—Three cases of neonatal septic osteo-arthritis due to umbilical artery catheterisation are described The clinical features were characteristic A week after removal of the catheter a septic osteo-arthritis developed The etiological agent in all three cases was Staphylococcus aureus resistant to penicillin The disease is probably caused by haematogenous spread of septic clots from thrombus formation on the catheter Symptoms and signs were few and vague It is suggested that septic osteo-arthritis due to umbilical catheterisation is more frequent than hitherto believed and low-grade forms might remain undiagnosed

KEY WORDS Newborn umbilical artery catheter septic osteo-arthritis osteomyelitis

Septic osteo arthritis seems to be a rare complication of umbilical artery catheterisation as only a single case has been reported previously (12) It has not been mentioned in surveys dealing with neonatal septic osteo arthritis and osteomyelitis (1 2 6 9 11) or in papers surveying the complications of umbilical artery catheterisation (3 4 8 10) Septic osteo arthritis in the newborn may be silent but resulting disablement is common (6 9 11)

MATERIALS

During the years 1974–76 3 cases of septic osteo-arthritis in infants below the age of 1 month were diagnosed A total of 60 umbilical artery catheterisations were performed during this period (2½ years) One of the following diagnostic criteria was fulfilled 1) existence of pus in the joint involved and growth of pathogenic bacteria or 2) existence of sterile pus in the joint but radiological signs of osteomyelitis in the metaphysis Table I presents the clinical findings in the 3 cases The clinical pictures were almost identical Pregnancy and birth were uncomplicated Because of IRDS (idiopathic respiratory distress syndrome) an umbilical artery catheter was inserted at the age of 4 hours in all 3 cases The following aseptic technique was observed The umbilical cord and the surrounding skin was cleansed twice with 2 5% iodine in alcohol The tincture was allowed to dry between the two applications The umbilical cord was cut 1 cm above the skin level and the catheter was inserted Sterile gloves sterile self adhering surgical drape and local application of 1% hexachlorophene powder were used Systemic or local antibiotic treatment was not given The umbilicus was covered by an appropriate sterile dressing and adhesive tape Following our experiences we later altered our technique to that used in surgical operative procedures with gown cap and mask The position of the catheter tip in the aorta on the level of the second lumbar vertebra was controlled by X ray Catheterisation lasted 3–7 days The first symptoms of osteo arthritis were noticed 3–10 days after removal of the catheter According to the literature skin infections or purulent rhinitis are frequent before septic osteo-arthritis (9) but this was not seen in our cases The first patient had a pleural tube inserted for 3 days because of pneumothorax the second and third patients were not exposed to procedures predisposing to infection other than umbilical artery catheterisation None was intubated for RDS The symptoms of osteo-arthritis were only local reduced mobility and tenderness of the involved leg which was kept in flexion In cases 1 and 2 there were no redness or swelling of the hip In case 3 there was swelling and warmth of the knee but no visible signs that the hip or ankle were affected 2 infants had no fever at all 1 had fever for one day All had high erythrocyte sedimentation rate (31–87 mm/h) and leucocytosis (18 000–25 000/μl) with a majority of neutrophils (48–73%)

Table 1 *Clinical and bacteriological findings in 3 cases of neonatal septic osteo arthritis*

Case no	Sex	Birth weight (g)	Clinical findings: Clinical diagnosis	Catheter inserted/ removed (day of life)	Arthritis symptoms (day of life)	Joints involved	Bacteriological findings: Catheter	Blood	Pus from joint
1	F	2 800	IRDS	2–7	14	Left hip	Staph aureus	No growth	No growth
2	F	2 500	IRDS	2–9	19	Left hip	Staph aureus	Not done	Staph aureus
3	M	3 000	IRDS	2–5	8	Right hip Left knee Right ankle	Not done	Staph aureus	Staph aureus

Antibiotics given for 1 day before joint puncture

Radiological findings At the time when the osteo arthritis was diagnosed X ray disclosed nothing abnormal in the 3 cases Two weeks later osteolytic processes in the metaphysis developed in cases 1 and 3 Case 2 developed lateral displacement of the femur but without signs of osteomyelitis In case 3 osteolytic processes developed in the distal metaphysis of the tibia without swelling or reduced mobility

Treatment The 3 patients were treated with joint puncture aspiration of pus immobilisation and lincomycin for 3–4 weeks The course was uncomplicated Signs and symptoms subsided and at a follow up 3–16 months later there were no clinical signs of sequelae X rays were normal

RESULTS

The bacteriological findings are presented in Table 1 In case 1 penicillin resistant Staphylococcus aureus was found on the catheter tip but there was no growth in pus from the hip joint after 1 day s antibiotic treatment In case 2 penicillin resistant Staphylococcus aureus phage type 3 A was found on the catheter tip and 19 days later in the joint In case 3 the catheter tip was not examined but blood culture and pus from hip and knee joints disclosed penicillin resistant Staphylococcus aureus with an identical pattern of resistance Phage typing was not done

DISCUSSION

The similar clinical features of the 3 cases strongly suggested a correlation between umbilical artery catheterisation and septic osteo arthritis After removal of the catheter the neonates had no signs or symptoms at all for 3 to 10 days, after which septic osteo arthritis developed The delay of local signs probably indicated that the haematogenous spread of infection from septic thrombosis was particularly related to the removal of the catheter It should be stressed that one of our patients showed Staphylococcus aureus phage type 3 A on the tip of the catheter and the same phage type was cultured 3 weeks later from plus aspirated from the hip joint

Neonatal septic osteo arthritis is generally caused by bacterial spread i e septic embolisation or transitory bacteraemia (2) Multiple bone or joint involvement often occurs (2) In neonates the disorder characteristically affects the metaphysis of long bones with a preference for the humerus and the femur (2 6) When umbilical catheterisation is involved the hip joint and the upper metaphysis of the femur seemed to be the most frequent localisation The inability of the metaphysis to handle infection is probably due to the sluggishness of the bloodstream and the non anastomosing small arteries in this area in which septic clots are readily captured In neonates however the infection can rapidly spread to the epiphysis Furthermore it has been pointed out that the joint capsule encloses the metaphysis of the hip joint which facilitates spread of infection to the joint (6) The distinction between septic

osteo arthritis septic arthritis and septic osteomyelitis is essential from a prognostic point of view The prognosis seems to be correlated to the age of the infant and the site and spread of the infection Lindblad et al (6) found that marked residual disability among children with acute haematogenous osteomyelitis was almost exclusively restricted to newborn infants with a complicating purulent osteo-arthritis of the knee or hip joint Radiological signs of osteomyelitis are often delayed In our cases we found a clinical picture of septic arthritis initially which later showed evidence of osteomyelitis in the metaphysis We have therefore called this condition septic osteo-arthritis We cannot exclude the possibility of a primary osteomyelitis in the metaphysis or epiphysis with spread to the joints

The majority of newborn infants have no significant surface bacterial colonization immediately after delivery Over the first days of life the skin and the umbilicus show a steady increase in numbers of bacteria It has been suggested that the umbilicus becomes colonized more quickly and perhaps more heavily than other parts of the skin There is thus a possibility that contamination of the catheter is more likely when catheterisation is performed at a later stage which may increase the risk of systemic infection

As demonstrated by aortography 95% of infants with umbilical artery catheters had distinct thrombus formation in the aorta (7) In several cases asymptomatic arterial clots in iliac and femoral arteries were found About 50% of indwelling umbilical catheters were contaminated with pathogenic or non pathogenic organisms (5) Therefore it seems reasonable to presume that septic embolism is a frequent occurrence It cannot be excluded that asymptomatic self limiting or low grade septic osteo arthritis due to umbilical catheters is a common event One of our patients demonstrated a septic osteo-arthritis without signs or symptoms and a silent but radiographically verified osteomyelitis

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Submitted April 25 1976
Accepted Aug 16 1976

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Acta Pædiatr Scand 66 229–232 1977

ANTIMICROBIAL FACTORS IN HUMAN MILK

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ABSTRACT Vinodini Reddy Bhaskaram C Raghuramulu N and Jagadeesan V (National Institute of Nutrition Hyderabad India) Antimicrobial factors in human milk Acta Paediatr Scand 66 229 1977.—Levels of Immunoglobulins lactoferrin and lysozyme were determined in milk samples obtained from well nourished and under nourished Indian women at different stages of lactation The concentration of immunoglobulins and lactoferrin was higher in colostrum than in mature milk while the lysozyme levels showed a progressive increase with the period of lactation There were no significant differences in the levels between the two groups of women Administration of iron did not alter either the total or percentage saturation of lactoferrin in milk These results indicate that antibacterial factors in milk are not influenced by the nutritional status of the mother and that iron supplementation does not interfere with the bacteriostatic function of lactoferrin

KEY WORDS Lactoferrin Immunoglobulins lysozyme

It is well known that the incidence of infections in bottle fed infants is higher than that in breast fed infants (9) Apart from increased bacterial contamination during bottle feeding this difference has been attributed to the presence of various antibacterial factors in breast milk such as immunoglobulins lysozyme and lactoferrin There is however little information regarding the changes in the concentration of these factors with the period of lactation and also whether the nutritional status of the mother modifies these levels We therefore decided to obtain such information

In vitro studies have shown that saturation of lactoferrin with iron abolishes its bacteriostatic activity This raises an important question as to whether supplementation of iron to the mother can alter the saturation of lactoferrin in milk and thus interfere with its biological function We have carried out studies to investigate this possibility

MATERIAL AND METHODS

Samples of breast milk were collected from 250 women at various stages of lactation The women were classified into two groups based on weight/height² index This index has been shown to reflect nutritional status (8) Women with an index of 0 18 or more were considered well nourished and those below 0 18 as undernourished Body weights ranged from 40–55 kg in the well nourished and 34–39 kg in the undernourished group Most of the well nourished women belonged to the high socio-economic group and were attending a private nursing home while the undernourished women belonged to the low socio-economic group and were attending the general hospital

Milk samples were analysed for IgA IgG IgM lactoferrin and lysozyme Immunoglobulins and lactoferrin were determined by the radial immunodiffusion technique (4) Purified colostral IgA and its specific antiserum were obtained from W H O reference centre for immunoglobulins and used for the estimation of IgA levels in milk The antiserum is specific for the secretory component of the molecule Lysozyme content of milk was determined by the method of Parry et al (11) Haemoglobin was determined by the oxyhaemoglobin method and serum albumin by the Biuret method

Eleven lactating women were given 200 mg of iron intramuscularly Milk samples were collected initially and

Acta Pædiatr Scand 66 229–232 1977

ANTIMICROBIAL FACTORS IN HUMAN MILK

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ABSTRACT Vinodini Reddy Bhaskaram C Raghuramulu N and Jagadeesan V (National Institute of Nutrition Hyderabad India) Antimicrobial factors in human milk Acta Paediatr Scand 66 229 1977 —Levels of immunoglobulins lactoferrin and lysozyme were determined in milk samples obtained from well nourished and under nourished Indian women at different stages of lactation The concentration of immunoglobulins and lactoferrin was higher in colostrum than in mature milk while the lysozyme levels showed a progressive increase with the period of lactation There were no significant differences in the levels between the two groups of women Administration of iron did not alter either the total or percentage saturation of lactoferrin in milk These results indicate that antibacterial factors in milk are not influenced by the nutritional status of the mother and that iron supplementation does not interfere with the bacteriostatic function of lactoferrin

KEY WORDS Lactoferrin Immunoglobulins lysozyme

It is well known that the incidence of infections in bottle fed infants is higher than that in breast fed infants (9) Apart from increased bacterial contamination during bottle feeding this difference has been attributed to the presence of various antibacterial factors in breast milk such as immunoglobulins lysozyme and lactoferrin There is however little information regarding the changes in the concentration of these factors with the period of lactation and also whether the nutritional status of the mother modifies these levels We therefore decided to obtain such information

In vitro studies have shown that saturation of lactoferrin with iron abolishes its bacteriostatic activity This raises an important question as to whether supplementation of iron to the mother can alter the saturation of lactoferrin in milk and thus interfere with its biological function We have carried out studies to investigate this possibility

MATERIAL AND METHODS

Samples of breast milk were collected from 250 women at various stages of lactation The women were classified into two groups based on weight/height index This index has been shown to reflect nutritional status (8) Women with an index of 0 18 or more were considered well nourished and those below 0 18 as undernourished Body weights ranged from 40–55 kg in the well nourished and 34–39 kg in the undernourished group Most of the well nourished women belonged to the high socio-economic group and were attending a private nursing home while the undernourished women belonged to the low socio-economic group and were attending the general hospital

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Eleven lactating women were given 00 mg of iron intramuscularly Milk samples were collected initially and

Table 1 *Antibacterial factors in colostrum and mature milk*
Figures in parenthesis indicate number of samples analysed

Group	Haemo globin (g/100 ml)	Serum albumin (g/100 ml)	Immunoglobulins mg/100 ml			Lysozyme (mg/100 ml)	Lactoferrin (mg/100 ml)
			IgA	IgG	IgM		
Colostrum (1–5 days)							
Well nourished women	11 5±0 37	2 49±0 065	335 9±37 39 (17)	5 9±1 58 (17)	17 1±4 29 (17)	14 2±2 11 (15)	420±49 0 (28)
Under nourished women	11 3±0 60	2 10±0 081	374 3±42 13 (10)	5 3±2 30 (10)	15 3±2 50 (10)	16 4±2 39 (21)	520±69 0 (19)
Mature milk (1–6 months)							
Well nourished women	12 8±0 43	3 39±0 120	119 6±7 85 (12)	2 9±0 92 (12)	2 9±0 92 (12)	24 8±3 41 (10)	250±65 0 (17)
Under nourished women	12 6±0 56	3 47±0 130	118 1±16 2 (10)	5 8±3 41 (10)	5 8±3 41 (10)	23 3±3 53 (23)	270±92 0 (13)

one month after administration of iron Saturated lactoferrin was determined by the method described by Bullen et al (2)

RESULTS

The results are shown in Tables 1 2 and Figs 1 and 2 The concentration of IgA was high in colostrum the mean level being 350 mg/100 ml and showed a rapid fall during the first 4 weeks of lactation reaching a mean level of 110 mg/100 ml in mature milk IgM showed a similar pattern The concentration of IgG was slightly higher in colostrum than in mature milk There were no significant changes in the levels of immunoglobulins during the subsequent stages of lactation

The concentration of lactoferrin was very high in colostrum the mean level being 600 mg/100 ml and showed a progressive fall up to 5 months of lactation and then stabilised at a mean level of 180 mg/100 ml Lysozyme content of colostrum was lower than that of mature milk and showed a progressive increase with the duration of lactation reaching the highest level of 42 mg/100 ml at 12 months

There were no significant differences in the levels of immunoglobulins lactoferrin and lysozyme between well nourished and under nourished women (Table 1) Also there was no correlation between these factors on the one hand and either Haemoglobin or serum albumin levels on the other

In women who received iron supplements initially the mean level of total lactoferrin was 240 mg/100 ml 9 0% of which was saturated There were no significant changes in the concentration of either total or saturated lactoferrin after administration of iron (Table 2)

Table 2 *Effect of iron therapy on lactoferrin in milk*
Values are means ±S E of 11 subjects

	Total lactoferrin (mg/100 ml)	Saturated lactoferrin (% of total)
Before iron therapy	240±29 0	9 0±7 15
After iron therapy	260±80 0	8 6±3 32

DISCUSSION

There are several clinical and epidemiological observations to suggest that breast milk has a protective role against infection in the infant Enteric infections due to *E coli* and *Shigella* are rare in breast fed infants unlike in artificially fed babies (5 7) Even in developing areas where exposure to various enteropathogenic organisms is common diarrhoeal disease is uncommon in breast fed babies

during early infancy and appears only with weaning (9) It is now recognized that there are many antibacterial factors in human milk which may be responsible for its protective function

Milk contains different classes of immunoglobulins such as IgG IgM IgA etc Of these IgA which is characteristic of bodily secre tions is the predominant immunoglobulin in human milk (6) Secretory IgA with antibody activity against many types of micro-organ isms confers local immunity in the gastro-intestinal tract of the infant The concentration of IgA is high in colostrum and shows a drop in the first week of lactational period But the increase in milk production may then com pensate for the fall in immunoglobulin con centration

Human milk also contains large quantities of an ironbinding protein lactoferrin which has a strong bacteriostatic effect (10) Bullen et al have found that saturation with iron abolished the bacteriostatic effect of lactoferrin (2) It has been suggested that iron therapy may interfere with the function of lactoferrin How ever the results presented here indicate that administration of iron to the mother does not alter the saturation of lactoferrin in milk and therefore may not interfere with its bacterio-static function This observation is of con siderable practical importance since iron supplements are currently being given to the

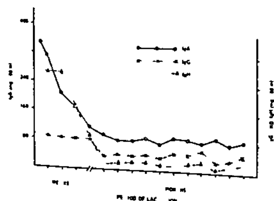


Fig 1 Immunoglobulin levels as mg/100 ml in milk secreted at different periods of lactation

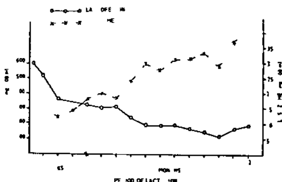


Fig 2 Levels of lactoferrin (O—O) and content of lysozyme (× ×) in milk secreted at different periods of lactation

pregnant and lactating women of poor com munities for the prevention of anaemia

Lysozyme is another antibacterial factor abundant in human milk (3) Its possible in fluence on the faecal flora is suggested by the fact that it is found in significant amounts in the stools of breastfed infants Unlike im munoglobulins and lactoferrin which showed highest concentration in colostrum lysozyme levels showed a progressive increase with the period of lactation Similar changes have been reported earlier in Indian women but not in western women (13 12) The reason for this difference is not clear

The results of the present study indicate that the antibacterial defense factors in milk are not influenced by the nutritional status of the mother This finding is of considerable public health significance since a majority of the women in poor communities are under nour ished These results are in line with the earlier studies reported from this Institute wherein the total protein content of milk samples ob tained from poor Indian women was found to be similar to that of well nourished western women (1)

The data presented here indicate that breast milk contains significant amounts of immuno-globulins lactoferrin and lysozyme even at the end of one year of lactation These qualities of breast milk may be of major importance for the infant s defense against infection par

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Acta Pædiatr Scand 66 233–235 1977

THE RADIAL DYSPLASIA/IMPERFORATE ANUS/VERTEBRAL ANOMALIES SYNDROME (THE VATER ASSOCIATION) DEVELOPMENTAL ASPECTS AND EYE FINDINGS

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ABSTRACT Say B Greenberg D Harris R. DeLong S L. and Coldwell J G (Department of Developmental Medicine & Child Neurology Children s Medical Center Tulsa Oklahoma USA) The radial dysplasia/imperforate anus/vertebral anomalies syndrome (the VATER association) developmental aspects and eye findings Acta Paediatr Scand 66 233 1977 —The developmental evaluations of four children of different age groups with radial dysplasia/imperforate anus/vertebral anomalies syndrome are presented These show that although the gross motor behavior is significantly delayed intelligence language and social development are within normal range Therefore the patients with this syndrome merit every effort toward rehabilitation Three of the patients discussed have ophthalmological abnormalities in addition to their major malformations It may be that congenital eye defects are another component of this syndrome of morphogenesis

KEY WORDS Radial dysplasia/imperforate anus/vertebral anomalies syndrome developmental studies eye findings

Since its description in 1968 under the title of polydactyly/imperforate anus/vertebral anomalies syndrome (3) the entity of multiple congenital malformations has been steadily expanded First as was predicted it was shown that the preaxial limb deformity was not always in the form of polydactyly but frequently consisted of hypoplasia or absence of the thumb (8) This was followed by reports indicating that other defects of mesodermal origin such as tracheoesophageal fistula with or without esophageal atresia cardiovascular and renal defects were not uncommon in these patients (4) Later in an attempt to name this condition various acronyms such as VATER association (2) VACTERL (1) etc were proposed which we do not favor for reasons described elsewhere (5) Finally it was suggested that despite the severity of their physical deformities these patients may have normal mental development (6 7) which was of course of great practical importance indicating that the subjects with this syndrome deserve all efforts toward rehabilitation

In this communication we present four new illustrative cases of different age groups confirming the favorable impression of others that children with this syndrome are not necessarily intellectually impaired Additionally we will propose that ophthalmological abnormalities may be another component of this syndrome of dysmorphogenesis

PATIENTS

Case I

G H a 14 month-old black female was the second born of presumably identical twin girls weighing 1125 g at birth while her twin weighed 1800 g The mother was ? years of age and was in good health The pregnancy was essentially uncomplicated except that it terminated

ticularly in developing countries where the risk of infection is high In poor Indian communities fortunately prolonged breast feeding is still the practice This should be encouraged as it has many advantages

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Submitted May 25 1976
Accepted Aug 16 1976

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the kidneys Although she had a II/VI mild systolic mur mur heard best at the apex the opinion of the cardiologist was that this was most likely a functional murmur Ophthalmologic examination showed her vision to be 20/30 on right and 20/40 on left correcting to 20/30 in each eye with refraction These findings were considered to be within normal range Psychological evaluation using the Stanford Binet Test revealed an I Q of 91 which indicated that the patient had a level of intellectual functioning in the lower end of the average range

Case 4

T N was a 10-year-old white male who at birth was found to have imperforate anus and esophageal atresia associated with tracheoesophageal fistula On the right forearm the radius carpal bones on the radial side as well as first metacarpal bone and the thumb were absent He had multiple deformities of the spine including a fusion of the first and second ribs on the right a hemi vertebra at the T1 and T2 level and spina bifida Also he was found to have a heart murmur possibly due to an ASD Ophthalmologic examination showed ptosis of the left eyelid anisocoria (right pupil larger than the left) and heterochromia iridis

A Wechsler Intelligence Scale for Children (Revised) was administered and his full scale I Q was found to be 100 His verbal I Q however was 109 while the performance I Q was 90

DISCUSSION

Using the Gesell Developmental Examination the adaptive behavior of Cases 1 and 2 appears in the low average or average range However gross motor behavior is significantly delayed and hypotonia appears in both children The third and fourth patients had I Q s well within the normal range and fine and gross motor development appeared adequate in Case 3 while it was slightly impaired in Case 4 It is very important to separate the gross motor delays seen in these youngsters from their adaptive function in order to prevent labeling them as mentally retarded especially in the earlier years and therapeutic efforts must be vigorous Considering the vast arrays of physical anomalies and their need for surgical correction and ongoing therapy every effort must be made to treat these children according to their intellectual levels rather than their motor function Also it is important to avoid making blanket statements regarding such children s ultimate intelligence when they are first evaluated in the neonatal period

Another interesting finding was the fact that three of the four children had unilateral ophthalmological abnormalities which had a tendency to appear in the left side (Table I) Most of the eye findings were of little clinical significance however no mention of ophthalmological findings could be encountered in other published cases This may indicate that such findings in our patients may be coincidental On the other hand one may speculate that the abnormalities related to the eye may have been missed because of their mild nature Future reports on similarly affected patients may shed a light on this aspect

ACKNOWLEDGEMENTS

We thank Dr R F Gates Mr L Warnberg for their assistance in evaluating their patients and Mrs B Barnes for her excellent technical help

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Submitted May 31 1976
Accepted Aug 11 1976

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Table 1 *Clinical findings*

R=right L=left Bil =bilateral ASD=atrial septal defect

Case	Age/Sex	Radial dysplasia	Imperforate anus	Vertebral anomalies	Eye findings	Others
1	14 mos/F	+	+	+	Ptosis (L) Internal strabismus (L) Cloudy cornea (L)	Renal dysplasia (?) Inguinal hernia
2	15 mos/F	+	+	+	Severe myopia (−8 diopters)	Renal dysplasia (?) 13 ribs (Bil) T E fistula
3	5 yrs/F	−	+	+	Vision 20/30 (R) 20/40 (L)	Renal dysplasia
4	10 yrs/M	+	+	+	Ptosis (L) Anisocoria Heterochromia iridis	T E fistula ASD (?)

at 35 weeks There were two amnions but only one chorion A previous child also was born prematurely weighing 1 600 g The mother denied exposure to drugs or irradiation during the pregnancy The family history was negative for birth defects

The patient was evaluated by us at the age of 6 weeks The spectrum of malformations observed included imperforate anus bilateral inguinal herniae a hypoplastic thumb on right and a left preauricular tag She also had hypoplasia of the left radius and ulna as well as multiple rib anomalies X ray of the spine showed hemivertebrae involving cervical vertebrae and L 1 Butterfly bodies were seen in the cervical and upper dorsal regions Some fusion also was present at the level of D 5 and 6 The heart was globular in shape but no definite abnormalities could be detected IVP showed faint visualization of both kidneys Ophthalmologic findings included ptosis of the left eyelid left internal strabismus and clouding of the left cornea without glaucoma (Table 1) The corneal lesion presented itself initially as a central erosion without edema which healed very poorly over a period of several months leaving a moderately dense corneal nebula No cause for this lesion could be established

She was evaluated using the Gesell Developmental Examination Because she was 5 weeks preterm her corrected age was 13 months The test results revealed an adaptive behavior 48 weeks gross motor behavior 28 weeks fine motor behavior 36 weeks language 40 weeks and personal social behavior 52 weeks Her adaptive behavior at 48 weeks while she was 57 weeks old gave her a developmental quotient of 84% which was in the low average range of intelligence Neurological examination demonstrated generalized hypotonia which was spontaneously improving in a cephalocaudad progression All reflexes appeared to be normal

Case 2

A M was a 15 month old female who was the only child born to a healthy couple The birth weight was 1 530 g The mother was 29 years of age and the father 31 at the time of the patient s birth The pregnancy which lasted 34 weeks was complicated by an episode of spotting about the 7th or 8th week and by massive edema and hypertension (240/150) during the last few weeks No history of exposure to known teratogens could be obtained Specifically she was not on any kind of contraceptive pills for the past 2 years prior to the pregnancy Family history was essentially negative especially with regard to birth defects

The patient s deformities in addition to imperforate anus included tracheoesophageal fistula with esophageal atresia hypoplastic right radius and 13 ribs bilaterally She also had 6 lumbar vertebrae and probable agenesis of the left kidney Ophthalmologic examination showed her right eye to be normal and her left eye −8 diopters myopic

This patient who was 6 weeks preterm considered at 58 weeks corrected age was tested using the Gesell Developmental Examination The results were as follows adaptive behavior 60 weeks gross motor behavior 48 weeks fine motor behavior 60 weeks language 16 months and finally personal social behavior 15 months Developmental quotient=60/58=103 was in the average intelligence range Neurological examination revealed hypotonia in all extremities and trunk which was improving without therapy in a cephalocaudad progression

Case 3

K M was a 5 year old black female born six weeks prematurely weighing 2 040 g Both mother and father were 21 years of age at the time of the patient s birth Exposure to drugs and irradiation during the pregnancy was denied The family history was essentially negative Two sisters of the patient 4 and 7 years of age respectively were reported as being in good health

The spectrum of malformations in the patients included imperforate anus with rectovaginal fistula hemivertebra at D-7 with a fusion of D-10 and D-11 synostosis of the 10th and 11th ribs on left and crossed fused ectopia of

Acta Pædiatr Scand 66 237–240 1977

CONTROL OF STREPTOMYCIN AND ISONIAZID IN MALNOURISHED CHILDREN TREATED FOR TUBERCULOSIS

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ABSTRACT Akbani Y Bolme P Lindblad B S and Rahimtoola R J (The Paediatric Clinics of Jinnah Postgraduate Medical Centre Karachi Pakistan and St Goran s Children s Hospital Stockholm Sweden) Drug control of streptomycin and isoniazid in malnourished children treated for tuberculosis Acta Paediatr Scand 66 237 1977 —In 12 malnourished children who were treated for tuberculosis plasma levels of streptomycin and isoniazid were followed Streptomycin was administered i m in a dose of 25–50 mg/kg/24 hours High initial plasma levels were reached (mean 44.3 μg/ml at 30 min) Streptomycin levels were followed for 5 hours and the mean plasma level at that time was 17 0 μg/ml From the present data a plasma half life of streptomycin of 3 5 hours has been estimated It is advised that streptomycin should not be given in doses above 25 mg/kg/24 hours to avoid potential toxic plasma levels especially if plasma levels cannot be measured It is also concluded from our study that renal function is not affected in malnourished children to an extent where streptomycin clearance is greatly affected Isoniazid was given orally 10 mg/kg/24 hours From 30 min to 6 hours after administration mean plasma levels of isoniazid above 0 5 μg/ml were observed In all children measurable plasma levels were obtained It is concluded that also children with malnutrition can absorb isoniazid after oral administration From our data it is suggested that the majority of the children in our study were rapid inactivators of isoniazid

KEY WORDS Tuberculosis children malnutrition streptomycin isoniazid

In the developing countries malnutrition is a dominating problem during early childhood Malnourished children seem to have a deficient cellular immune system (3 4 18) which makes them susceptable to several infectious diseases in particular tuberculosis An adequate chemotherapy is therefore critical for a successful treatment of tuberculosis in malnourished children

However in the state of malnutrition it may be difficult to predict the fate of pharmacological agents since drug absorption (8) as well as drug metabolism (15 16) and excretion (1 12) may be different in a malnourished child as compared to a well nourished child A combination of streptomycin and isoniazid is commonly used in the treatment of tuberculosis By studying the pharmacokinetics of isoniazid and streptomycin in malnourished children treated for tuberculosis we intended to gain some knowledge of an adequate dosage in this group of children At present the therapy is based only on studies of well nourished children

MATERIAL AND METHODS

Included in the study are 12 children 5 months–6 years of age admitted to the Pediatric Clinic Jinnah Postgraduate Medical Centre Karachi and treated for tuberculosis with streptomycin and isoniazid The routine treatment during the initial phase of serious tuberculosis was streptomycin sulphate in citrate buffer (pH 5 8–6 1) 25–50 mg i m /kg

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[1] Natr citrat 14 g natr sulfis 7H O 4 7 g acid citric ca 1 g (pH 5 8–6 1) methylparaoxibenz 1 5 g propylparaoxibenz 0 ? g aqua steril ad 1000 g

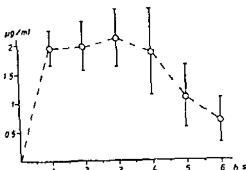


Fig 2 Plasma levels of isoniazid after oral administration of 10 mg/kg of INH to 12 children with malnutrition Means±S E M are shown

tion was almost reached at one hour and was then kept for the next three hours

DISCUSSION

Surprisingly little is known about the pharmacokinetics of streptomycin in well nourished children and nothing at all is studied about this drug in children with malnutrition It is reported that hearing loss can occur in children to mothers treated with streptomycin during pregnancy (5 17) and in adults it is well known that streptomycin can damage the function of the eighth cranial nerve and cause especially vestibular dysfunction (14) Thus vertigo nausea etc might serve as warning signals of too high streptomycin concentrations However in sick children such symptoms may be difficult to notice and therefore the risk of permanent damages to the eighth cranial nerve with loss of hearing and labyrinthine dysfunction are increased In the present study we have not been able to follow those children who survived to test for hearing abnormalities

From the study of Line et al (14) it seems as if high peak levels of streptomycin are to a lower degree associated with side effects than a retarded clearance of the drug This would indicate that patients with decreased renal function are at risk to develop ototoxicity Premature infants are known to have decreased renal function and it has been reported that the plasma half life of streptomycin in premature children was 7 hours compared to adult values of 2 4–2 7 hours (7) The mean plasma half life value in our group of patients was estimated to 3 5 hours and from these results it does not seem as if children with marasmus as a rule have a greatly affected renal function However three of our children of which two had received 50 mg/kg had concentrations of streptomycin at 5 hours exceeding 25 µg/ml (28–39 µg/ml) It would therefore seem important to determine streptomycin plasma levels not only as was done in the present experiments during five hours after the dose but also just before the next dose (i e 24 hours after administration of streptomycin)

It seems advisable from our data to use no more than 25 mg/kg/dose unless streptomycin concentrations can be determined In children with signs of impaired renal function it is necessary to observe possible side effects as nausea vomiting nystagmus etc It is not satisfactory to relay on simple renal tests like creatinine or urea in states of malnutrition since these tests may fall within normal values even when renal function is low

Measurable concentrations of isoniazid were reached in all our patients after oral administration The malnutrition did not seem to affect the gastrointestinal absorption in these marasmic infants

Isoniazid is metabolized in the liver before excretion and the main metabolite is the acetyl derivative There exist at least two populations of people in the ability of metabolizing isoniazid slow and rapid inactivators due to different concentrations of activities of the enzyme (6 11) The proportion of slow/rapid acetylators seem to vary in different ethnical populations (6) From our experiments where the drug was given orally it is not possible to draw definite conclusions about the rate of metabolism of isoniazid since absorption can still continue even though the plasma concentration curve is declining However the relatively rapid drop in plasma concentration from 4 to 6 hours (half life≈90 min) makes it reason

Table 1 *Patients treated with streptomycin and isoniazid*

Initials	Sex	Weight (kg)	Height (cm)	Age (y)	Comment	Hb (g%)	MAC* (cm)
MT	M	7 0	64	1	Slight oedema on feet	9 0	10
SR	M	7 0	–	2 3/12	–	8 4	9 5
KK	F	5 8	67 5	1 1/12	Pale and dehydrated	9 0	–
I	M	6 5	–	1 6/12	T B meningitis	8 6	8 5
BB	F	5 0	60	5/12	T B meningitis	10 2	10
AQ	M	6 0	–	1 3/12	–	8 5	–
NF	M	5 8	–	1 6/12	–	10 0	9 0
AS	M	6 7	69	2	–	–	–
N	M	6 0	–	1 9/12	–	5 4	9 0
MY	M	5 5	–	4	Grossly malnourished	6 5	9 0
RB	F	10 5	103	6	Grossly malnourished	–	9 0
A	M	9 2	–	1 6/12	Pale but active	–	13 5

* Mid arm circumference

once daily and isoniazid orally 10 mg/kg once daily Capillary blood samples were taken at several time intervals after drug administration The individual patients are listed in Table 1 All children were in a state of serious malnutrition

For the assay of streptomycin plasma was put on microdiscs 10 µl on each disc usually in triplicates The discs were dried and frozen and were later transported in frozen and dried condition to Stockholm Sweden where the analysis was made

The microbiological agar diffusion method was used for the determination of streptomycin calculated as the base A staphylococcus strain was used in the assay and a freshly prepared standard series was always included when the determinations were done A more detailed description of the method has been published by Jalling et al (9) and Bolme & Eriksson (2)

Plasma was also frozen for the determination of isoniazid and transported to Stockholm The method described by Lever (13) was used for isoniazid assay The method is based on a condensation in aqueous solution of aromatic acid hydrazides and β-diketones to form yellow anions in the presence of a strong base We have modified the method so that 100 µl of plasma was enough for a valid determination of concentrations down to 0 15 µg/ml of free isoniazid

The blood samples were collected during a two month period They were frozen immediately after the tests The assays of streptomycin were performed 3–12 weeks after the sample collections and no decomposition occurs during this time All isoniazid analyses were done during a period of one week 4–6 months after the tests

RESULTS

Fig 1 illustrates the concentrations of streptomycin from 30 min to 5 hours after the administration of streptomycin High initial concentrations were achieved The mean value for the entire material at 30 min was 44 3 µg/ml and the highest individual value was 72 µg/ml (after 50 mg/kg streptomycin) The highest individual value after 25 mg/kg was 52 0 µg/ml Even at 5 hours after streptomycin administration plasma values above what is considered as therapeutic levels (10 µg/ml or more) were achieved the mean value in all children being 17 0 µg/ml An estimation of the plasma half life of streptomycin from the present material gives a value of 3½ hours No acute side effects of streptomycin were noticed

The plasma concentrations of isoniazid after a single oral dose of 10 mg/kg are illustrated in Fig 2 In all patients a measurable plasma concentration was achieved The peak concentra

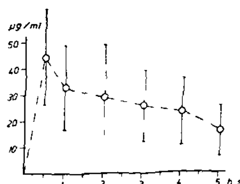


Fig 1 Plasma levels of streptomycin after i m administration of 25–50 mg/kg of streptomycin sulphate in citrate buffer (pH 5 8–6 1) to 12 children with malnutrition Means±S E M are shown

Acta Pædiatr Scand 66 241–242 1977

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MATERIAL AND METHODS

CDH was diagnosed in 3? new born girls between 1956–1961 No cases with combined malformations were included (teratological cases) and in all cases the treatment had resulted in completely normal hip joints The girls were *all* born in the city of Malmö by Swedish parents By the time of the interview all the girls diagnosed during this time period had had their menarche In addition 288 girls from the City of Malmo schools were studied these did not have congenital hip dislocation and were born by Swedish parents in Malmo The control girls were born during the same time period as the CDH sample *With the* aid of the City School Medical Service the girls to be

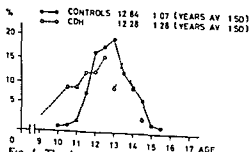


Fig 1 The distribution of menarcheal age in CDH girls and control girls There is a skewedness to the left in the CDH girls

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Submitted July 2 1976
Accepted Sept 9 1976

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MATERIAL AND METHODS

CDH was diagnosed in 37 new born girls between 1956–1961 No cases with combined malformations were included (teratological cases) and in all cases the treatment had resulted in completely normal hip joints The girls were all born in the city of Malmo by Swedish parents By the time of the interview all the girls diagnosed during this time period had had their menarche In addition 288 girls from the City of Malmö schools were studied these did not have congenital hip dislocation and were born by Swedish parents in Malmo The *control girls* were born during the same time period as the CDH sample With the aid of the City School Medical Service the girls to be

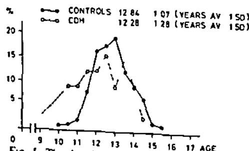


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MATERIAL AND METHODS

CDH was diagnosed in 32 new born girls between 1956–1961 No cases with combined malformations were included (teratological cases) and in all cases the treatment had resulted in completely normal hip joints The girls were all born in the city of Malmo by Swedish parents By the time of the interview all the girls diagnosed during this time period had had their menarche In addition 288 girls from the City of Malmo schools were studied these did not have congenital hip dislocation and were born by Swedish parents in Malmo The control girls were born during the same time period as the CDH sample With the aid of the City School Medical Service the girls to be

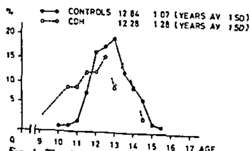


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Acta Pædiatr Scand 66 241–242 1977

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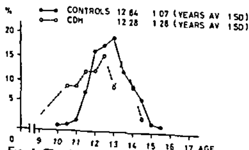


Fig 1 The distribution of menarcheal age in CDH girls and control girls There is a skewedness to the left in the CDH girls

interviewed as controls were selected from several schools to represent a socio-economic cross section of the city All the girls CDH girls and controls were interviewed in the same manner with regard to their age at the time of the first menstrual bleeding

RESULTS

The age at menarche for the 32 girls with CDH was about 6 months less than for the control sample The difference was significant $0.01>p>0.001$ (t test of samples of unequal size) (Fig 1) The distribution of menarcheal age in the control sample demonstrated in the figure hardly deviates from normal there may be a slight tendency to skewedness to the right In the CDH sample, however there is a skewedness to the left, whereas the modal values for the two sets do not differ significantly This seems to indicate the presence among the CDH cases of a small number of girls with an exceedingly early menarche

The seven CDH girls who were 11 or less at the time of menarche were compared with the total sample of CDH girls diagnosed in the years 1956–1964 (6) In four of the seven girls there was at least one additional case of CDH in the family as compared with 7% in the total sample (98 girls) Heights and weights were available in six of the seven girls In all instances the heights and the weights were above average for CDH girls

DISCUSSION

In the control sample which was drawn in order to match the CDH cases rather than to describe the distribution of menarcheal age in Malmo there exists a certain bias owing to the presence among the youngest girls interviewed of a few who had not yet attained their menarche Since the number is small and taking into account the previously known age distributions of menarcheal age, this fact could at most deduct 0 1 year from the real average menarcheal age It is possible to draw the conclusion that at present the average menarcheal age in the city of Malmo is less than 13 which supports the concept of a continuing secular trend of decrease

The findings of the present study support the hypothesis of a hormonal influence in CDH Additional support for this hypothesis are the findings of Fredensborg (6) of a somewhat increased height and weight in young girls who had been born with CDH as compared with a control sample In the present study those girls who had the lowest menarcheal age deviated even more being taller and heavier than not only control girls but also other CDH girls Both factors menarche and body size reflect an early physical maturation

ACKNOWLEDGEMENTS

Financial support was obtained from the Swedish Medical Research Council (project no B76-17X 2737-08A) and from the Herman Jarnhardt Foundation Olof Smitt M D Chief School Physician helped with the collection of the control samples

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Submitted May 14 1976
Accepted Sept 6 1976

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Acta Pædiatr Scand 66 243–246 1977

HERPES ENCEPHALITIS

B WOLMAN and M LONGSON

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ABSTRACT Wolman B and Longson M (Department of Paediatrics and North Manchester Regional Virus Laboratory Booth Hall Children s Hospital Manchester England) Herpes encephalitis Acta Paediatr Scand 66 243 1977.—Four cases of herpes encephalitis treated with dexamethasone and cytarabine Cases were all verified by brain biopsy and immunofluorescent techniques Attention is called to the need for early diagnosis but the difficulty of deciding when to perform brain biopsy is discussed Early treatment with effective drugs is necessary to prevent permanent brain damage

KEY WORDS Herpes encephalitis brain biopsy treatment long term brain damage

It has been estimated that up to 50 cases of herpes encephalitis are diagnosed each year in the United Kingdom (8) but that perhaps as many as 150 cases are unrecognized Although it has been suggested (9 12) that mild herpes encephalitis with a good prognosis can occur many have argued (8) that the overall mortality is as high as 60% There is a grave danger of neurological crippling in survivors (10) We report the cases of four children who have survived but whose mental condition is severely affected

CASE REPORTS

Case 1

K B a female was admitted to hospital on 7 April 1974 aged years 9 months with a one week history of a bad cold with nasal discharge Four days before admission she had started with fits initially rightsided becoming progressively more severe and generalized On the day of admission she became confused and disorientated There were no significant neurological features on examination The CSF showed 64 leucocytes mainly polymorphs per mm but the chemistry was normal and no organisms were seen on the stained slide or obtained on culture The Mantoux test was negative An EEG originally showed bilateral high amplitude delta activity throughout maximal in the left frontal region Two days later the record changed showing periodic high delta waves on a relatively flat background The fits continued despite paraldehyde and diazepam—the girl remaining unconscious A diagnosis of encephalitis was made but as there was no improvement in the next few days a brain biopsy was performed on 11 April 1974 The immunofluorescent test for herpes simplex antigens was positive and eventually *Herpesvirus simplex* was isolated on tissue culture The child was treated with cytosine arabinoside 10 mg per kg daily intravenously for five days and also given dexamethasone 1 mg per kg load followed by ? mg 6-hourly for four days Convulsions continued from time to time The child required diazepam and chlorpromazine to control the fits for the next four months but they eventually stopped and treatment was discontinued She has remained in the same vegetative state with spastic quadriplegia requiring feeding and total care by the parents now twelve months after the illness

Case 2

S K a boy was admitted on 16 August 1974 aged 13 years 10 months with a history of dizziness and diarrhoea starting on 0 July This settled after three or four days but on 10 August he was feverish dizzy and he vomited four times He became progressively drowsy and confused with visual hallucinations but no fits He had been given penicillin for four days of these days by his general practitioner On admission he was confused unable to co-operate showing no specific clinical abnormalities The CSF showed 66 leucocytes 70% of which were polymorphs No other abnormalities were noted in

the CSF and no organisms were seen on the stained slide or obtained on culture A viral encephalitis was diagnosed The EEG showed diffuse slow wave activity most prominent over the left hemisphere particularly in the left temporal region On 17 August brain biopsy was performed *Herpesvirus simplex* antigens were detected by immunofluorescent tests and the virus was eventually isolated on tissue culture He was treated with cytosine arabinoside 10 mg per kg daily intravenously for five days and dexamethasone 10 mg per kg load followed by 10 mg 6-hourly for four more days Treatment was started the day after admission but probably at least seven days after the onset of the illness and maintained for five days Convulsions started on the day after admission and he became unconscious Despite sedation with diazepam and paraldehyde convulsions occurred from time to time and he required tube feeding His condition remained stationary till December 1974 but in January 1975 he gradually regained consciousness stopped convulsing and over the ensuing months he has become more interested in his surroundings although speech has not returned and he cannot stand or walk He will not talk although he does seem to understand a great deal of what is said to him His admission to a Junior Training Centre may be possible

Case 3

A R a female was admitted to another hospital on 24 January 1975 aged 7 months having had a generalized convulsion after a six hour history of crying refusing feeds and vomiting On admission she was unconscious with mainly left sided convulsive movements and weakness of the left arm Examination of the CSF showed 75 cells per mm^3 equally polymorphs and leucocytes Protein was slightly raised but no organisms were seen on the stained slide or obtained on culture A bacterial or viral meningitis was considered and treatment was started with intravenous ampicillin The Mantoux test was negative For 48 hours the convulsions recurred The child remained semi conscious and febrile On 26 January she seemed to improve became conscious and afebrile but the following day the convulsions recurred On 28 January she was transferred to this hospital in a semi conscious state showing convulsive movements of the left side with hemiparesis An EEG showed generalized hyperexcitability mainly in the left temporal region with secondary subcortical activity The diagnosis of herpes encephalitis was suspected A brain biopsy was done and *Herpesvirus simplex* antigens were detected by immunofluorescent tests and the virus itself was later isolated on tissue culture Treatment with cytosine arabinoside 10 mg per kg daily was given intravenously for five days and dexamethasone was given as a 1 mg per kg load followed by 2 mg 6-hourly for four days The convulsions were controlled with diazepam and phenytoin By mid February her condition was unchanged she had occasional convulsive movements was semi conscious and was unable to suck or swallow She had another episode of status on 1 March after which she developed a spastic quadriplegia sucking and swallowing with difficulty Over the past three months her condition has remained unchanged she takes no interest in her surroundings and she screams when disturbed Long term management is difficult because of domestic and social problems

Case 4

M J W a female was admitted to hospital on 22 November 1974 aged 9 months with a history of two weeks vague ill health being fretful and miserable On the day of admission she had a right sided fit and was found to have a right paresis and looked obviously ill There were no other signs The CSF showed 18 white cells 14 of which were lymphocytes with a normal chemistry Bacterial examination was negative and the presumptive diagnosis was viral meningitis with the possibility of a space occupying lesion The baby was observed for four days during which there was slow but obvious deterioration with increasing fits and wildly oscillating temperature and conjugate left deviation of the eyes On 26 November 1974 the EEG showed widespread abnormality with slow wave activity A brain scan on the same day revealed two areas of abnormal uptake of isotope (over vertex and over left occiput) On the following day the carotid angiogram produced a remarkable picture of venous blushing with a profusion of new vessel formation in the left parietal and temporal regions A clinical diagnosis of herpes encephalitis was now queried and a brain biopsy obtained from the affected parietal area The specimen yielded *Herpesvirus simplex* by immunofluorescence and culture The infant was treated with dexamethasone 4 mg intramuscularly initially and 2 mg intramuscularly every 6 hours thereafter and with cytosine arabinoside 100 mg intravenously daily by single bolus injection for five days Initially there was no improvement in her condition but a fortnight after starting the anti viral drug the patient began to improve and over the next twelve months appeared to become more and more interested in her family

Subsequent neuro-psychological assessment of the child has however revealed that she has been left with a most severe disability Now twelve months after her illness she is a severely retarded child and is unable to communicate Using the Griffiths Mental Development Scale she shows a comparatively high loco-motor profile but displays poor performance scores There is nothing in her play activity to suggest a normal development and although she has regained a substantial degree of physical function she has not kept up her cognitive ability to act purposefully

DISCUSSION

These four children illustrate the different and bizarre ways in which herpes encephalitis can present The clinical diagnosis of encephalitis is always difficult When the infection is with *Herpesvirus simplex* the presentation seems to be more varied with fits vomiting diarrhoea

paresis of the cranial nerves clouding of consciousness and variation of severity over a period of several days

The clinician cannot make the diagnosis unaided CSF changes are non specific The EEG may show gross changes but even these are not diagnostic although high wave complexes against a background of diffuse slowing particularly in the temporal lobes has been claimed as a special feature Immunofluorescent demonstration of viral antigens in brain biopsy specimens is now a reliable test giving a result within 1–3 hours from receipt of the specimen but this implies brain biopsy which also may yield the virus on culture within 24–48 hours

The problem therefore seems to be one of deciding when to carry out a brain biopsy in a suggestive case Many children who have repeated convulsions with fits for 24–48 hours settle subsequently without trouble Brain biopsy is certainly not the first investigation to be considered In the cases here reported four days after admission to this hospital (5–12 days after the onset of disease) two days after admission to hospital (8 days after onset) four days after admission (5 days after onset) and four days after admission (about 2½ weeks after onset) were the times before brain biopsy was done Is therefore a period of three or four days before brain biopsy is considered the earliest possible time one can accept?

There is no doubt that the mortality of the disease can be influenced by treatment (3 5 11) but the form which this treatment should take is a matter of dispute

Herpes encephalitis in its severe form causes a disastrous progressive necrosis of the brain It is reasonable to suggest that treatment whatever form it may take can only be effective if it is applied as early as possible during the destructive process

There is clinical and neuropathological evidence (3 4 6) that patients die from cerebral oedema If specific virus chemotherapy is to be effective this oedema must be controlled at all costs and there must be aggressive cerebral decompression This decompression may be achieved surgically or medically with steroids (such as dexamethasone) Most authorities now appear to be agreed that dexamethasone should be administered in high doses in all cases of herpes encephalitis but it remains controversial whether the steroid should be covered by the simultaneous administration of an anti viral drug In the case of herpes encephalitis there are three specifically antiherpes drugs in use—idoxuridine cytarabine and vidarabine

The use of idoxuridine has now been discontinued because of substantial toxicity and failure to prevent death (2) Vidarabine is a relatively new anti viral drug which is currently undergoing clinical assessment in the United States and which has been the subject of preliminary and optimistic reports

Cytarabine is like idoxuridine and vidarabine potently active against the replication of *Herpesvirus simplex* experimentally in cell culture Preliminary promising reports concerning its use come from the Herpes Encephalitis Working Party (7) which should report in 1976 or early 1977 A disquieting feature of the use of cytarabine is the severe neurological damage found in survivors

Previous authors have frequently commented that survival from herpes encephalitis may be associated with severe damage to the brain In view of the pathological process involved and of the intense disruption of nervous tissue which is known to occur this is not surprising The destructive process should be interrupted at a very early stage Earlier diagnosis and earlier exhibition of dexamethasone and cytarabine might improve the prognosis However in our case No 3 both diagnosis and treatment were established on the 5th day of illness and it is difficult to see how this can be improved upon It could also be argued that an invasive diagnostic approach involving brain biopsy might lead to an increased incidence of neurological sequelae but brain biopsy in competent hands is a safe procedure The temporal lobe is the site usually used

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Acta Pædiatr Scand 66 247–253 1977

REVIEW ARTICLE

LEARNING DISORDERS

Some Medical Aspects

NEIL GORDON

From the Royal Manchester Children s Hospital
and Booth Hall Children s Hospital Manchester England

ABSTRACT Gordon N (The Royal Manchester Children s Hospital and Booth Hall Children s Hospital Manchester England) Learning disorders—some medical aspects Acta Paediatr Scand 66 247 1977.—The first skills are motor ones and these depend on perceptual development and the organisation of movement If development is deviant the child will be clumsy The clinical picture is considered and the effects this may have in the school situation Disorders of language development may be secondary to other factors such as deafness or brain injury or may be a specific disability The various grades of the latter are described Both perceptual and language disorders can underlie reading retardation and the analysis of a particular child s difficulties is stressed The etiology of these conditions is discussed with particular reference to the failure of integration Intracerebral connections may not form be destroyed or not used The role of the Doctor is an important one and this includes not only diagnosis and assessment but also helping the child in the home and school especially when emotional and behaviour complications occur The doctor must also act as a questioner and co-ordinator

KEY WORDS Perceptual-motor disorders disorders of language development reading retardation etiology the role of the doctor

Learning disorders are common and there must be few people who can honestly say they have not had some difficulties of this kind In Britain over six per cent of 7 to 8 year old children appear to have a very significant de gree of perceptual motor handicap (3) and 1 6 per cent of boys and 0 8 per cent of girls aged 7 have almost unintelligible speech soon after starting school (14)

PERCEPTUAL MOTOR DISORDERS—CLUMSY CHILDREN

Among the first skills to be learnt are motor ones These depend on the appreciation of shape and sizes the relationship of parts to the whole the orientation of objects and other aspects of perception If these are difficult to learn movements will be clumsy and motor skills difficult to acquire If the disability persists as the child grows older reading and writing will be affected If it is hard for the child to interpret the shape of a triangle this will also apply to letter shapes and will delay the ability to read at least for a while

Some clumsy children will apparently have no particular troubles with perception but cannot easily organise their movements Any one when acquiring a new skill will be clumsy until they practise and build up a repertoire of memory of movements and if an instrument is involved until this is part of the body image It

CONCLUSION

We report four cases of proven herpes en cephalitis all treated with cytarabine who survived the disease We are however severely disquietened by the sequelae and we wonder whether anti viral drugs in herpes encephalitis may be encouraging the survival rate at the price of producing severe neurological cripples

In these four children the poor results of treatment of this disease may be due to irreversible brain damage occurring before the diagnosis is made or to the failure of drugs currently available If diagnosis cannot be achieved earlier then we are entirely reliant on effective drugs in the management of a case at any stage of the illness

ACKNOWLEDGEMENTS

Our thanks are due to Dr M J Noronha for allowing us to include case 4 and to Dr C M P Bradstreet of the Standards Laboratory London for the supply of reagents used in the diagnosis of our patients We also acknowledge the very competent technical assistance provided by Mr A S Bailey F I M L T

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Submitted April 12 1976
Accepted Sept 15 1976

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is this process that some children seem to find particularly difficult Studies on acquired cerebral lesions suggest that right hemisphere lesions are *likely* to cause perceptual dis abilities and left hemisphere lesions defects of motor organisation (2) When it comes to therapy distinctions of this kind are important Those with predominantly perceptual dis abilities will need to have the task broken down into component parts and demonstrated, with full use being made of the child s verbal ability It is always easier to discriminate if you describe the task in words If the defect is in motor organisation presumably the solution does lie in continual *practice, suitably moti* vated

Children with perceptual motor disabilities seem to be fairly numerous As has been mentioned, (3) about 6% of 7 to 8 year old children have difficulties of these kinds which means *1 to 2 such children in* each class of 30 to 40 If their difficulties are not recognised they can easily be accused of not trying when in fact they have been doing so to a greater extent than the average Then it is not surprising that they develop secondary emotional disturbances Their difficulties will not only be in the classroom because of illegible writing and so on but these are likely to involve out of class activities as well They are often poor at games and no one wants them in their team or to go on holiday with them Over activity and poor concentration are often associated with inco ordination of this kind and if such school difficulties arise the child is likely to become depressed and to withdraw from the situation

Unless the disability is very marked it may not cause particular comment or put the child under pressure before school entry Also for the majority of these children progress at school will most probably be satisfactory if they can be given a certain amount of help and understanding and above all, credit for their efforts which will have to be greater than aver age So much of the teaching that takes place in the good infant class is just what these children need Therefore, with few exceptions the best time to screen children for this disabil ity may be at school entry or soon afterwards Then the teacher can be alerted to the possibil ity of a child s difficulties and encouragement of efforts and a certain amount of special help may be all that is required There will be some children who are so severely affected that they will need to be educated in a special class or even a special school for example a school for physically handicapped children

DISORDERS OF LANGUAGE DE VELOPMENT

Acquiring one s mother tongue may not be thought of as a learnt skill It is true that the brain has in the words of the linguists the propensities to enable it to extract from the linguistic input the relevant data needed to organise *language into a consistent system* but the same concept could be applied to many other skills (12) There can be no doubt that environment will affect the richness and com plexity of language and the ability to express one s feelings and ideas These statements ap ply to the normal child and if there are specific difficulties in language development the need for teaching language is even more evident

The acquisition of speech and language may be delayed as the result of a variety of dis abilities some of which can be treated It may be possible to compensate for environmental factors for example in preventing some of the deprivation suffered by mentally and physi cally handicapped children A child with se vere cerebral palsy will live in a very restricted world perhaps consisting of one or two rooms at home and the classroom at school There will be few opportunities of exploring the world and experimenting in order to build up the concepts on which so much of inner lan guage depends It has been shown that the way parents talk to their mentally handicapped child can be over simplified and deprive them of the wider experience of language on which

children depend to increase their vocabulary (4) Handicapped children must have the world brought to them if they cannot explore it themselves and must be given the opportunity for new experiences to the limit of their capacities

Deaf child plus hearing aid does not equal normal child Children with severe peripheral deafness as their only handicap still have dif ficulties with abstract thought even if success fully treated from an early age Presumably this is related to the faulty auditory input and the difficulty in explaining to such children ideas rather than facts

Severe emotional disturbances in early life can affect language development but in the case of autism the very abnormal behaviour pattern is most probably the result rather than the cause of the severe disorder of language function The basic problem for these children may be a profound difficulty in using symboli sation of any kind (16)

Acquired disorders of language develop- ment in childhood present special problems at least if the child is affected before the age of ten Not only will there be the results of the damage to the brain in terms of loss of func tion but also an interference with the continu ing development of language function This is well illustrated in Charles Dickens account of Laura Bridgman whom he met in 1842 (10) It is a very entertaining account of how she was helped after becoming blind and deaf and it emphasises that spoken language is the final stage of this development and the importance of what occurs before the child uses words If she had not begun to use words before her illness it is most unlikely that she would have accomplished what she did It may help par ents to realise this and that the first word a child says with meaning is the beginning of the last stage of language development It emphasises the importance of speaking to a child in as meaningful a situation as possible without asking much in return for long periods of time

When the development of language function appears to be delayed to a much greater degree than other aspects of development and in the absence of secondary causes such as those discussed it can be regarded as a specific dis order As with any such disorder the majority will be mildly affected but as Peckham (14) has shown speech and language disorders are fre quent enough among children in Britain start ing school The speech of 14 4 % of boys and 9 7% of girls is not fully intelligible at school entry and to stress this again among 1 8% of boys and 0 9% of girls at this time most of their words are unintelligible

The majority of children with specific dis orders of language development as with any other disability will improve and if given a certain amount of help and understanding in their early years will overcome their disabili ty if they have average intelligence As the severity of the disability increases sounds other than those of language may lack mean ing and the term congenital auditory im perception has been used to describe such children Although fortunately rare the audi tory agnosia can be even more profound and then the child may not respond to sounds of any kind and the abnormal auditory response thought to be due to peripheral deafness The diagnosis may be particularly difficult because a number of causes such as anoxia at birth may damage the auditory nucleus in the brain stem as well as causing more widespread brain damage and central and peripheral deafness may co-exist

Even when the auditory agnosia is severe the abnormal response can alter as the child grows older The child will begin to react to sound and this may be the result of the neces sary associations being made between differ ent parts of the cortex so that meaning can be given to the electrical stimuli coming from the peripheral auditory receptors a theme which will be considered later on

Although the majority of children with specific disorders of language development can be helped within the normal school some times in a special class with the teacher and

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these causes severe brain damage producing gross mental handicap or obvious forms of cerebral palsy and the lesser degrees of dam age leading to the more subtle disabilities of specific learning disorders The fact that boys are more often affected than girls may be ex plained by the message of the Y chromosome in delaying development as suggested by Oun sted & Taylor (13) If the vulnerability of the brain to damage is directly related to its imma turity and cerebral maturation is slower in boys than girls they will be more at risk They will also have a longer exposure to genetic information good and bad

Possible reasons for failure of integration

Improvement of learning disorders un doubtedly occurs as the child grows older but some degree of disability can remain How ever with increasing age it does become easier to avoid or circumvent the difficulties This stresses the importance of not only trying to help the child with remedial teaching but also *finding* out the child s assets and concentrat ing on these as well so that success is assured in some fields

Also it may mean that the number of con nections made between neurones in the brain is more important than the actual number of cells up to a point anyhow Association seems to underlie so much of learning which is com forting as one grows older as it should mean that you can go on learning to an advanced age even if the neurones are becoming pro- gressively fewer

If this is part of the explanation why do these connections fail? Maybe they are not formed they can be destroyed and sometimes they are not used Dobbing (6) has *shown the* importance of adequate nutrition during the major spurt of brain growth whenever this oc curs In humans it begins soon after mid pregnancy so that if the placental circulation is inadequate during the last trimester the de veloping foetus will be deprived of adequate nutrition and the dendritic connections which grow at such an enormous rate at this time will be stunted Anoxia during pregnancy may be a more frequent cause of brain damage than anoxia at birth and Towbin s (17) work strongly supports this But whenever it occurs connections within the brain may be irrepara bly damaged

There is some evidence in the case of vision anyhow that function exerts an effect on den dritic connections and in maintaining them Interference with input from one eye which prevents integration at a cortical level can have a profound effect on synaptic morpho- logy (8) If this is so to what extent can learn ing affect cortical structure?

There is another factor which may contri bute to difficulties of learning at least among the older children and that is inhibition If a stimulus does not have any particular meaning or interferes with perception in some way or other it will be inhibited A classical example is the amblyopic eye of the squinting child If the same happens in the auditory field why should there not be lazy ears? If auditory stimuli are inhibited in this way it will obvi ously interfere with language development and it may be as important to keep alive an awareness of sound in a child with a severe disorder of this kind as it is to fit a hearing aid to a peripherally deaf child

THE ROLE OF THE DOCTOR

What is the role of the Doctor in all this particularly the hospital-based doctor? He is not an educator and should not presume to advise on teaching techniques but he has a *role to play* and a very important one Many aspects of this are obvious The importance of diagnosing causes and seeking to prevent them has already been stressed Among children with handicaps the disabilities are usually multiple but even now high tone deaf ness is sometimes missed among children with delayed language development and a severe refractive *error* among children with visual perceptual problems Also there are dangers in

speech therapist working together in the classroom there will be a few children who may need the kind of expert help that can only be provided in the special school The method used as Moor House School by Mr Lea (9) is a good example of this with the use of colours to identify nouns verbs and adjectives This seems better than using a predominantly audi tory approach when that is the sensory chan nel most severely affected

READING RETARDATION

Difficulties in learning to read are relatively common although the cause varies widely It seems pointless to become emotional about terminology when it now seems to be generally recognised that there is a group of children who for one reason or another have difficulty in learning to read and spell and are in great need of help

Rutter (15) has defined reading backward ness as an attainment in reading accuracy and comprehension 28 months or more below chronological age and reading retardation a specific disability, as reading accuracy and comprehension 28 months or more below the predicted level on the basis of the child s age and I Q on a modified W I S C

The former can be due to such causes as lack of schooling inappropriate teaching emotional disturbances defective vision and low I Q Children having specific difficulties in learning to read (dyslexia) can be divided into several main groups (1), and it is obviously important to analyse the child s disability be fore deciding on how best to help The largest number of children have a disorder of language function (dysphonetic) They have difficulties with spoken speech which after a while they usually overcome only to find it abnormally difficult to read There is a defect in symbol sound integration They recognise words globally and cannot sort out and blend compo nents Spelling is by sight not by ear

A much smaller group have severe perceptual difficulties (dyseidetic) and can truly be called word blind They cannot perceive letters and words as configurations They are poor spellers but their mistakes are not bizarre Some of them will be clumsy children Children who have both types of dis order will be in great trouble

There are some children who do not fit neatly into any of these groups Some seem to have a very specific defect of visual recogni tion They have no difficulties with spoken language development and are not clumsy They just cannot recognise certain visual symbols correctly They can spell a word ver bally without difficulty but not when they write it down Others have very specific de fects of sequencing and synthesis

This is the kind of information which is needed for planning remedial teaching and it seems logical to base this on the use of unaf fected channels of sensory input whole word techniques for the audio phonic group a phonetic approach for the visuo spatial group

ETIOLOGY OF LEARNING DISORDERS

If these learning difficulties are so frequent it is particularly important to study causes and at tempt to prevent these disabilities Hereditary factors obviously play a part but may have been over emphasised in the past even in the case of dyslexia Barton Childs (5) could pro duce relatively little evidence in favour of ge netic factors determining these learning dis orders It therefore seems likely that the ma jority are acquired disabilities and therefore possibly preventable Studies including one in Manchester (unpublished) on clumsy children favour the kind of pre peri and postnatal disorders which result in brain damage partic ularly hypoxia before and during birth This concept is supported by Drillien s (7) studies in which she found that a third of children born with a birthweight of 2 000 g or less were hand icapped and that in a quarter of these there was evidence of intrauterine hypoxia It seems that a spectrum of disabilities results from

these causes severe brain damage producing gross mental handicap or obvious forms of cerebral palsy and the lesser degrees of damage leading to the more subtle disabilities of specific learning disorders The fact that boys are more often affected than girls may be explained by the message of the Y chromosome in delaying development as suggested by Ounsted & Taylor (13) If the vulnerability of the brain to damage is directly related to its immaturity and cerebral maturation is slower in boys than girls they will be more at risk They will also have a longer exposure to genetic information good and bad

Possible reasons for failure of integration

Improvement of learning disorders undoubtedly occurs as the child grows older but some degree of disability can remain However with increasing age it does become easier to avoid or circumvent the difficulties This stresses the importance of not only trying to help the child with remedial teaching but also finding out the child s assets and concentrating on these as well so that success is assured in some fields

Also it may mean that the number of connections made between neurones in the brain is more important than the actual number of cells up to a point anyhow Association seems to underlie so much of learning which is comforting as one grows older as it should mean that you can go on learning to an advanced age even if the neurones are becoming progressively fewer

If this is part of the explanation why do these connections fail? Maybe they are not formed they can be destroyed and sometimes they are not used Dobbing (6) has shown the importance of adequate nutrition during the major spurt of brain growth whenever this occurs In humans it begins soon after mid pregnancy so that if the placental circulation is inadequate during the last trimester the developing foetus will be deprived of adequate nutrition and the dendritic connections which grow at such an enormous rate at this time will be stunted Anoxia during pregnancy may be a more frequent cause of brain damage than anoxia at birth and Towbin s (17) work strongly supports this But whenever it occurs connections within the brain may be irreparably damaged

There is some evidence in the case of vision anyhow that function exerts an effect on dendritic connections and in maintaining them Interference with input from one eye which prevents integration at a cortical level can have a profound effect on synaptic morphology (8) If this is so to what extent can learning affect cortical structure?

There is another factor which may contribute to difficulties of learning at least among the older children and that is inhibition If a stimulus does not have any particular meaning or interferes with perception in some way or other it will be inhibited A classical example is the amblyopic eye of the squinting child If the same happens in the auditory field why should there not be lazy ears? If auditory stimuli are inhibited in this way it will obviously interfere with language development and it may be as important to keep alive an awareness of sound in a child with a severe disorder of this kind as it is to fit a hearing aid to a peripherally deaf child

THE ROLE OF THE DOCTOR

What is the role of the Doctor in all this particularly the hospital based doctor? He is not an educator and should not presume to advise on teaching techniques but he has a role to play and a very important one Many aspects of this are obvious The importance of diagnosing causes and seeking to prevent them has already been stressed Among children with handicaps the disabilities are usually multiple but even now high tone deafness is sometimes missed among children with delayed language development and a severe refractive error among children with visual perceptual problems Also there are dangers in

allocating a child to a school catering for a particular disability and forgetting that he may have several significant handicaps For example, it is not uncommon for deaf children to have perceptual motor disorders which in their own right would seriously interfere with progress at school The doctor has an important role to play in analysing the child s difficulties Labels may serve their purpose but what one really wants to know is the exact nature of the disability so that a remedial programme can be planned As Kinsbourne (11) has said, if you ask a child to do something and he does not, you can ask again, and on the third occasion you can shout at the child, or work out the components of the request, ask about them separately, and find out which one is not understood This analysis will be much more effective if the doctor is a member of a team with help from the psychologist, speech therapist and physiotherapist

These learning difficulties are no particular respecter of the intelligence level, and especially if there is a degree of mental retardation a realistic view of the child s potential must be made It may help the parents to accept this and the need for special school if the situation is fully discussed and it is pointed out that one of the greatest disservices you can do to a child is to put him in a school situation which *is not meaningful to him*

There are a number of adverse situations at home and at school which the doctor may be able to modify especially with the help of a social worker The children are often under excessive pressures for instance they may spend most of their waking hours doing lessons, or they may be falsely accused of not trying when they are really trying much harder than the average

Emotional problems are an especial concern of the doctor This often involves the whole family and the problems can easily get out of proportion particularly in families with learning high on their list of priorities The doctor can often discuss these problems as someone outside the immediate situation He can point out that there is rarely an ideal solution, and that sometimes the school is doing all it can to help, although of course this is not always so Again apart from helping the child with the learning difficulties he can try and make sure that the child succeeds in some way

The doctor must be in the forefront of those pressing for more efficient services After the diagnosis and assessment he can act as a *questioner, checking on the child s progress* and on the kind and effectiveness of any special help that is given Above all he is in an ideal position to act as a co ordinator of all the various experts that may be needed and he must interpret and explain the various opinions to the parents

Learning difficulties affect many children They are not necessarily referred because of these but may come to the clinics with complaints such as headaches abdominal pain tics and nocturnal enuresis If the disabilities are not recognised for what they are early in school life emotional complications are almost inevitable and these in their turn will delay progress Much of this is preventable and although children can often overcome their difficulties at what cost emotionally and intellectually The failure of potential is difficult to measure but is likely to be large Doctors must play a greater part in helping *these children and if possible as members* of assessment teams whether these are based in hospitals or in the community

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Submitted Aug 12 1976
Accepted Oct 8 1976

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These learnıng dıfficultıes are no partıcular respecter of the ıntellıgence level, and espe cıally ıf there ıs a degree of mental retardatıon a realıstıc vıew of the chıld s potentıal must be made It may help the parents to accept thıs, and the need for specıal school ıf the sıtuatıon ıs fully dıscussed and ıt ıs poınted out that one of the greatest dısservıces you can do to a chıld ıs to put hım ın a school sıtuatıon whıch ıs not meanıngful to hım

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The doctor must be ın the forefront of those pressıng for more effıcıent servıces After the dıagnosıs and assessment he can act as a questıoner checkıng on the chıld s progress and on the kınd and effectıveness of any spe cıal help that ıs gıven Above all he ıs ın an ıdeal posıtıon to act as a co ordınator of all the varıous experts that may be needed and he must ınterpret and explaın the varıous opın ıons to the parents

Learnıng dıfficultıes affect many chıldren They are not necessarıly referred because of these but may come to the clınıcs wıth com plaınts such as headaches abdomınal paın tıcs and nocturnal enuresıs If the dısabılıtıes are not recognısed for what they are early ın school lıfe emotıonal complıcatıons are almost ınevıtable and these ın theır turn wıll delay progress Much of thıs ıs preventable and although chıldren can often overcome theır dıfficultıes, at what cost emotıonally and ıntellectually The faılure of potentıal ıs dıf ficult to measure but ıs lıkely to be large Doctors must play a greater part ın helpıng these chıldren and ıf possıble, as members of assessment teams whether these are based ın hospıtals or ın the communıty

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Submitted Aug 12 1976
Accepted Oct 8 1976

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Acta Pædiatr Scand 66 255-256 1977

CASE REPORT

A CASE OF FOCAL DERMAL HYPOPLASIA (GOLTZ SYNDROME) WITH SOMF NEW ASPECTS

N BEGANOVIĆ and E J P LOMMEN

From the Paediatric Department St Joseph Ziekenhuis Eindhoven the Netherlands

ABSTRACT Beganovic N and Lommen E J P (Paediatric Department St Joseph ziekenhuis Eindhoven the Netherlands) A case of focal dermal hypoplasia (Goltz syn drome) with some new aspects Acta Paediatr Scand 66 255 1977.—A case of Focal Dermal Hypoplasia (Goltz syndrome) diagnosed at birth is reported Some findings not formerly described (hemimelia schizis of the palatum molle and the absence of one um bilical artery) are reported Normal findings in chromosome studies with banding techniques are discussed

KEY WORDS Focal dermal hypoplasia Goltz syndrome X linked dominant inheritance single umbilical artery hemimelia

Focal dermal hypoplasia as a nosological en tity was first described by Goltz et al (1) Up till now 50 cases have been reported in the literature All patients show dysplasia of the skin and several other ectodermal structures Mesodermal structures also are frequently affected Because of the dysplastic character of the syndrome Ishibashi & Kurihara (4) pre fer to name it dysplasia rather than hypoplasia

In the following we shall describe a case diagnosed at birth and associated with some defects not formerly described by other au thors Chromosome investigations by means of banding techniques were carried out

CASE REPORT

The patient a girl was born in October 1975 as the first child of healthy young parents Pregnancy was unevent f l There were no hereditary disorders or congenital malformations in the family The child's weight was only 1960 g (small for date) The placenta was small (375 g) and had only one umbilical artery

Several malformations were noticed (Fig 1) The oc ciput was flat with a short neck There were low set and malformed ears The external auricular canal was normal as were the eardrums There were severe colobomas of the iris the chorioidea and the retina of both eyes and a bilateral microphthalmia There was a short mandibula a broad nasal bridge and flattening of the left side of the face the mouth appeared to be small The gingivae were hypertrofic and showed several elevations with a bony consistence

The uvula was absent and the palatum molle was split The palatum durum was intact as were the maxilla and the lips

The skin showed small spots in a linear arrangement in which the dermis was lacking resulting in herniation of subcutaneous fat through the defects

The neck the right shoulder the abdomen and the buttocks showed larger areas with a complete lack of dermis and epidermis presenting as red decubitus like lesions (Fig 2)

A skin biopsy of the affected areas showed micro scopically a thin and locally absent cutis with fatty tissue reaching up to the epidermis without overlying elastic fibers There was a large umbilical herniation (diameter 4 cm)

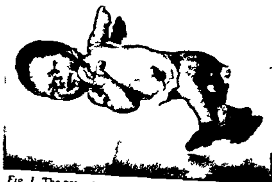


Fig 1 The patient s appearance at 1 day of age

Acta Pædiatr Scand 66 257–260 1977

CASE REPORT

SINUS HISTIOCYTOSIS WITH MASSIVE LYMPHADENOPATHY

K BROSTRØM and U BAANDRUP

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Kommunehospitalet Aarhus Denmark

ABSTRACT Brostrøm K and Baandrup U (University Department of Paediatrics and University Institute of Pathology Kommunehospitalet Aarhus Denmark) Sinus histiocytosis with massive lymphadenopathy Acta Paediatr Scand 66 257 1977 —The present case report brings to attention an unusual form of massive benign lymphadenopathy which can simulate malignant lymphoma The disease occurs mainly in children and is characterized by a protracted course with painless enlargement of the cervical lymph nodes fever leucocytosis mild anaemia raised erythrocyte sedimentation rate and hypergammaglobulinaemia The diagnosis is confirmed by a quite distinct microscopic picture of the involved lymph nodes SHML is considered to be an unusual response to an infection in an individual with abnormal host defence though this is not confirmed by the investigations undertaken in the presented case

KEY WORDS Sinus histiocytosis benign lymphadenopathy

An unusual form of histiocytosis was first described by Azoury & Reed in 1966 (1) Recently Rosai & Dorfman have analysed 34 cases and have established the disorder sinus histiocytosis with massive lymphadenopathy (SHML) as a clinical and pathologic benign entity (4 5) Most cases of SHML occur in children and are characterized by painless cervical lymphadenopathy often bilateral and of massive proportions Other lymph node groups are sometimes involved A few patients had involvement of soft tissue of the orbit and skin and of lymphoid tissue in the nasopharynx The children are usually in a good general condition Concomitant features are mild fever leucocytosis with neutrophilia anaemia markedly elevated ESR and hypergammaglobulinaemia

The pathologic picture of a lymph node biopsy is quite distinct with capsular and pericapsular fibrosis The normal lymph node architecture is totally disintegrated the sinuses being distended and packed with histiocytes showing pronounced lymphophagocytosis

The lymphadenopathy regresses slowly over years with complete recovery in most cases The aetiology and pathogenesis are not known It is suggested that SHML is the expression of an infection in an immunologically deficient individual (2 3 5)

CASE REPORT

A previously healthy girl developed at the age of 3½ years painless enlargement of the cervical lymph nodes There was no history of a preceding sore throat or febrile episode

At admission her general condition was good During the first few days she ran a low grade fever of maximum 38 5 C On the left side of the neck there was considerable enlargement of lymph nodes (Fig 1) The remaining superficial lymph node groups were unremarkable but roentgenological examination showed moderate enlarged



Fig 2 Cutaneous lesion at the right arm

The left forearm and hand were completely absent and the left upperarm showed a distal fingerlike appendix only consisting of soft tissue The right hand showed a low set thumb and only three fingers and resembled a lobsterclaw There was slight syndactyly of the 4th and 5th toe of the left foot and of the 2nd and 3rd toe of the right foot X ray examinatin showed hypoplasia of the right clavicle There were no rudimentary rests of the bones of the left forearm and hand and the left humerus showed a forkshaped distal ending From the right hand one finger was completely missing Skull vertebrae and other bones seemed to be normal

Intravenous pyelography showed torsion of both kidneys with rather large but otherwise normal pyela

There was no significant elevation of a virus antibody titer and immunoglobulins were found to be normal for age Chromosome studies were performed A normal female karyogram was found in the standard techniques (50 cells counted 7 cells analysed) and also in the banding techniques in an analysis of 6 cells in G banding and Q banding no aberrations were found Especially both X chromosomes appeared to be normal

DISCUSSION

The symptomatology of this child correlates completely with the syndrome of focal dermal hypoplasia as described in older children first by Goltz et al (1) and subsequently by Gorlin et al (3) Ishibashi & Kurihara (4) and Valerius (5) The last author gives a very comprehensive review of the findings at birth in his patient and there is a striking similarity between his description of the epithelial defects in his patient and our findings whereas all other patients described at a later age show a partial repair of these defects

Three findings in our patient have not formely been described

1 The hemimelia found in our patient is unique, other patients show only digital absences or partial aplasia of the forearm (4)

2 No case history mentions the number of umbilical vessels and as Valerius (5) also reports the birth of an affected girl who was small for date the findings of one umbilical artery in our case could be of significance as well in relation to the complexity of symptoms as to the cause of the small for date birth weight

3 Valerius (5) describes a patient with a cheilo gnato-palatoschizis Our patient shows only a schizis of the palatum molle and absence of the uvula

Whereas the focal dermal hypoplasia is supposed to be inherited in an X linked dominant way with a lethal course in male patients (2) several authors have searched in vain for aberrations in the X chromosome (2 4 5 present report) using the standard techniques In addition in our study no abnormality could be detected in the chromosomal substructures as revealed by means of the recently developed banding techniques

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Submitted May 17 1976
Accepted Aug 11 1976

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CASE REPORT

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Submitted May 17 1976
Accepted Aug 11 1976

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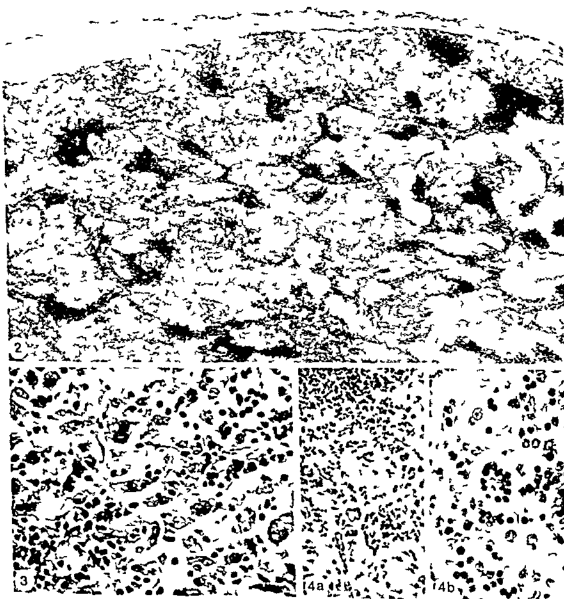


Fig ? Enlarged lymph node with thickened fibrous capsules and marked dilation of sinuses occupied by histio c tes (H E 5)

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Fig 4 (*a*) Lymphophagocytosis of histiocytes (H E × 1 5) (*b*) The gland like arrangement of lymphocytes in a hi tiocyte (H E x 300)

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Fig 1 Patient at admission The lymphadenopathy was not at its height

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Investigations ESR 136 mm/hr Hb 9 2 g/100 ml WBC 11 6×10⁹/l with 80% neutrophils platelets 660×10⁹/l Bone marrow aspirate showed hyperplasia of the myeloid series and moderate eosinophilia but no abnormal cell forms were present Serum GOT 24 K U Total skeletal X ray was normal A lymph node biopsy gave the diagnosis of reactive hyperplasia Serologic tests for ornithosis toxoplasmosis salmonellosis brucellosis streptococcosis mononucleosis and Ebstein Barr virus were done all with negative results Cultures from the throat and lymph node biopsy were negative for bacteria and fungi A PPD skin test was negative Serum contained normal concentrations of IgA IgM and IgE while IgG concentration was markedly elevated (18 9 g/l) Diphteria and tetanus antitoxin titers were both extremely elevated 2 years following active immunization There was a negative LE preparation a slightly positive test for antinuclear antibodies and a normal serum complement C3 Cellular immunity measured in vitro by lymphocyte transformation experiments showed normal response to plant mitogens and to allogenic lymphocytes The NBT test showed normal levels before and after stimulation with various antigens

Although no obvious infectious aetiology was found she was treated with oxacillin for 2 weeks but with no benefit The lymph nodes continued to enlarge and another biopsy was done

Pathological examination The material consisted of 6 fused lymph nodes each measuring 2–4 cm with intact fibrous thickened capsules On sectioning the tissue was firm and homogeneous The colour was yellowish Microscopic examination revealed capsular and pericapsular fibrosis with sparse lymphocyte and plasma cell infiltration Normal lymph node architecture had disappeared The sinuses were markedly distended by histiocytes (Fig 2) Most of the histiocytes had vesicular round nuclei with a distinct nucleolus and abundant finely vacuolated or granular cytoplasm The cell outline was smooth Some cells had a typical foamy cytoplasm and these cells were often placed subcapsulary and/or clumped together Several areas showed atypical histiocytes with hypercromatic nuclei large nucleoli and multinucleation (Fig 3) The histiocytes had pronounced cell phagocytosis particularly of lymphocytes (Fig 4*a* and 4*b*) and to a lesser extent of erythrocytes When numerous the lymphocytes showed a tendency to distribute themselves around the cytoplasm of the histiocytes in a gland like pattern (Fig 4*b*) Mitoses were not frequent and eosinophilia necrosis and granulomas were not seen

Only a few lymph follicles and germinal centres remained The compressed intersinusal tissue contained lymphocytes and plasma cells In some areas plasma cells were quite numerous eventually binucleated and containing Russel bodies

Special stains a small amount of diastase labile material was found as was a moderate amount of neutral lipids Hemosiderins were seen in some areas especially in the medullary cords Stains for acid fast bacteria fungi and parasites were negative

The histological picture was consistent with SHML

Subsequent course The patient has been followed for 1 year She has received no therapy except for antibiotics before establishment of the diagnosis The lymphadenopathy has been regressing during the last 4 months She is no longer anaemic and ESR has decreased to 29 mm/hr Her condition has all the time been excellent

DISCUSSION

The present case is typical for SHML As in the majority of cases so far described in the literature SHML was not diagnosed at admission In fact the pathological picture described from the first biopsy as reactive hyperplasia was identical with SHML diagnosed in the second biopsy

The suspected clinical diagnosis in most cases as in ours is malignant lymphoma Nevertheless the malignant lymphomas including the syndrome of histiocytic medullary reticulosis and other conditions such as histio

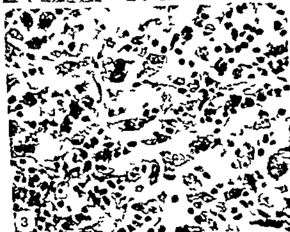




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Acta Pædiatr Scand 66 261–263 1977

CASE REPORT

INSULIN AND GROWTH HORMONE SECRETION IN A LEUKAEMIC GIRL WITH HYPOTHALAMIC SYNDROME

G SCHILIRÒ A RUSSO A SCIOTTO and R VIGO

From Paediatric Clinics I and II University of Catania Catania Italy

ABSTRACT Schiliro G Russo A Sciotto A and Vigo R (Paediatric Clinics I and II University of Catania Catania Italy) Insulin and growth hormone secretion in a leukaemic girl with hypothalamic syndrome Acta Paediatr Scand 66 261 1977.—The authors report a girl with acute lymphoblastic leukaemia presenting hypothalamic syndrome characterized by meningeal leukaemia hyperphagia and obesity Insulin and growth hormone secretion studied with arginine and insulin stimulation tests showed a high peak of serum insulin and no response of growth hormone Insulin and growth hormone responses to these tests reverted to normal after intrathecal methotrexate

KEY WORDS Insulin growth hormone leukaemia hypothalamic obesity

The central nervous system (CNS) involvement during acute leukaemia is one of the most important problems in the treatment of this disease Hypothalamic hyperphagia is a rare form of CNS involvement first described by Sansone (18) in 1954 Since then many other cases have been reported (1 2 3 4 5 10 11 13 ?0 21) but all the reports are limited to the description of the clinical picture and the pathological findings without endocrine studies Only in one case (3) a decreased level of thyrotrophic hormone and absence of gonadotrophic activity has been reported

We have studied the insulin and growth hormone (HGH) secretion in a leukaemic girl with hypothalamic hyperphagia and obesity before and after the successful intrathecal treatment with methotrexate (MTX)

CASE REPORT

A 10-year-old girl was admitted into our clinic in October 197 because of pain in the leg pallor and low grade fever Bone marrow aspiration revealed a typical picture of acute lymphoblastic leukaemia Chest X ray revealed no mediastinal involvement Treatment was started with triamcinolone (100 mg/m²/day orally) and vincristine (1 5 mg/m²week i v) and complete remission was achieved after four weeks The patient was discharged with maintenance therapy of 6-mercaptopurine (50 mg/m²/day) and MTX (10 mg/m²/twice a week) In March 1973 the patient presented CNS symptoms but bone marrow remained in complete remission Treated with orthovoltage cranial irradiation (1?00 rads) and low doses of intrathecal MTX (10 μg/kg) at first daily and thereafter every –3 days (14) the patient achieved complete remission after 3 weeks Further administration of intrathecal MTX was very irregular because the parents often broke the appointments In July 1974 at the age of 11 years and 8 months the patient was readmitted because she had had hyperphagia and excessive weight gain for three weeks Physical examination showed cushingoid face obesity and minimal meningeal signs Height was 140 cm (mean for her age 138 cm) weight was 56 kg (mean for her age 31 kg) The bone marrow was in complete remission Skull X ray EEG and fundoscopy were normal Skeletal age was also normal Lumbar puncture showed an increased pressure of the spinal fluid and the presence of 12 0 blast cells/ml The patient was treated with intrathecal MTX (5 mg/m /week) and her appetite rapidly decreased The spinal fluid was clear after the second injection of MTX and the girl lost 10 kg within one month

person with immunological deficiency (2 3, 5) This may be the fact but we are unable to confirm it by the investigations done in the presented case

Our patient was treated with antibiotics but without success as is the experience from other cases Some patients have been submitted to cytostatics corticosteroids and/or radiotherapy without benefit at best they have not been harmed (5) Given the now established benign nature of SHML it can be concluded that no specific therapy is warranted and that a re assuring approach about the self limited nature of the condition be given to the parents

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Submitted June 14 1976
Accepted Aug 27 1976

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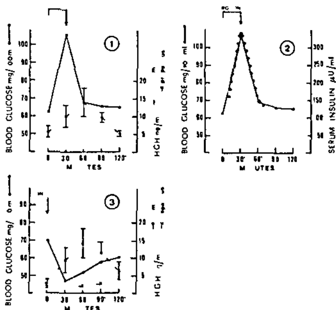
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Submitted July 7 1976
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Figs 1–3 Arginine and insulin tolerance tests before treatment of the hypothalamic syndrome

METHODS

Arginine tolerance test L Arginine hydrochloride in saline solution (0 5 g/kg) was administered intravenously during 30 min

Insulin tolerance test Regular crystalline insulin was *injected intravenously at the dosage of 0 1 units/kg*

In both tests blood samples were obtained at 0 30 60 90 120 min The serum was separated at 4 C and stored at −20 C until assayed The blood glucose was measured by a specific glucose oxidase method serum insulin and HGH were determined in triplicate by standard radioimmunoassay techniques (9 19) The tests were performed before treatment with intrathecal MTX and three months after the recovery

Nine normal children (3 males and 6 females) of age ranging from 10 to 12 years were tested as controls

RESULTS

The graphs 1–5 report the results of these studies The arginine tolerance test before treatment produced an increase of the blood glucose during the infusion which then gradually dropped to the normal values The serum insulin showed a very high peak whereas the HGH was *completely unresponsive* (Figs 1 and 2) The insulin tolerance test showed also a normal response of blood glucose and no response of HGH (Fig 3) Control subjects showed responses between the normal range in both tests (Figs 1–3)

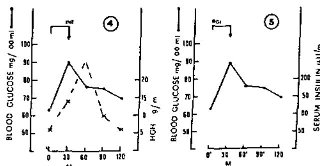
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DISCUSSION

The main sign of the observed syndrome is hyperphagia which distinguishes it from the common leukaemic meningoencephalitis and *obesity is caused by this excessive appetite* The hypothalamic infiltration by leukaemic cells has been demonstrated in some of the reported cases of the syndrome (4 5 18 21) Because of the normal appearance of the hypothalamic nuclei in some cases it has been suggested that even the increased spinal fluid *pressure with dilatation of the third ventricle* may mechanically produce a hypothalamic injury (17)

A low level of HGH with decreased response to the stimulation tests has been found both in experimental obesity (8 15) and in the obesity of adults and children (6 7 16) The *study of HGH in our patient demonstrates that* the response in hypothalamic obesity is similar to that reported in other types of obesity The identical behaviour of HGH in obesity of different aetiology the rapid normalization of the basal HGH level and the normal response to the stimulation tests after treatment and *weight reduction support the opinion that* the decreased secretion of HGH is a secondary effect of obesity

However in our case we can not rule out



Figs 4 and 5 Arginine tolerance test after treatment and recovery

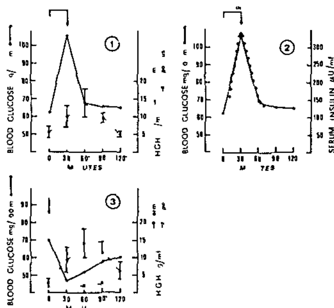
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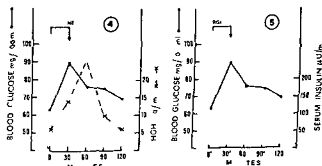
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Acta Pædiatr Scand 66 265 1977

LETTER TO THE EDITOR

On Palpation of the Femoral Pulse in the Newborn

Sir

Most textbooks in pediatrics (1 2 4) and in pediatric cardiology (3) rightly stresses the importance of including palpation of periferal pulses as part of the physical examination of any child

However most medical students and junior doctors and in fact not so few pediatricians find it somewhat difficult and timeconsuming to palpate the femoral artery This is especially true for the routine examination of the newborn at the maternity ward where several babies are screened and the doctors time schedule often tight The difficulties encountered are probably due to the palpation technique used a technique which the doctor or student originally learnt during his first basic training in physical examination on adults The use of the tip of indexfinger may be useful in the adult but is difficult in the crying and struggling baby The textbooks cited above offer no description of technique to be used



The grip and procedure illustrated (Fig 1) has proven to be useful The baby s right thigh and knee is grasped gently with the investigator s left hand and the tumb placed in the groin With this grip the thumb is automatically placed parallel to and over the long axis of the femoral artery and the pulse is readily located The grip is similar to the one used in the Barlow manoeuver when testing for congenital hip dislocation and the two tests could therefore be performed in connection with each other

According to tradition it is thought to be wrong and unprofessional to use the thumb when palpating but my own experience and that of others who have been introduced to this technique have convinced me that this is an exeption to that rule

B Bjarke

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ANNOUNCEMENT

The Czechoslovak Medical Society J E Purkyne and The Czechoslovak Pediatric Society will organize from July 4 to 6 1977 the 19th Pediatric Congress with International Participation The main topics of the Congress are

1 Nourishment of the suckling and the infant
2 Malabsorption syndrome Digestive enzymes
3 Diencephalohypophyseal system Adrenal cortex
4 Diagnostic and therapeutic actualities

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An unselected population of 1 567 four year-old children

KEY WORDS Pre-school children, caries, gingivitis

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1 Brecher G & Stohlman, F., Jr Humoral factors in erythropoietin. *In* L. M Tocantins & R. Penn (eds.) *Progress in hematology* Grune & Stratton, New York 1959 p 110.
2 Smith, C. A. *The physiology of the newborn infant* Thomas, Springfield Ill. 1967 3rd ed., vol. 2, p 120
3 Vedra B & Ulrych, J., Anaerobiosis in normal and asphyxiated premature newborns 2. Four approaches. *Acta Paediatr Scand 49 129 1960.*

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BOOK REVIEWS

Peter M Jeavons & Graham F A Harding Photo*sensitive epilepsy* 121 pp illus *Clinics in Developmen*tal Medicine No 56 Spastics International Medical Publications W Heinemann Medical Books Ltd London 1975 £4 50

The authors a clinician (P J) and a scientist (G H) have written an excellent book on the sensitivity to natural or artificial flickering light which is an interesting epileptic phenomenon In the first part of the book there is a review of the literature about clinical aspects of photosensitivity laboratory studies with photostimulators and EEG responses to photic stimulation and therapy in photosensitive epilepsy The second part deals with clinical and laboratory studies of 454 photosensitive patients referred to the EEG Departments of Dudley Road Hospital and the Children s Hospital in Birmingham (U K) between 1961 and 1973 The series included 332 patients with fits provoked by the flickering light of daily life and 122 cases with fits not triggered by light but whose EEGs showed abnormalities during photic stimulation in the laboratory The onset of their photosensitive epilepsy was before 17 years of age in 79% of the cases with a maximum around puberty Of all patients 63% were females Watching T V induced fits in 299 cases 30 of them were impulsively attracted to the T V screen Systematic and interesting investigations of clinical and EEG responses to light flashes with various physical properties were performed in order to develop a standardised technique for photic stimulation and elucidate the mechanism behind photosensitivity The chapter on therapy deals with the avoidance of stimulus conditions which may provoke fits the use of sodium valproate as anticonvulsant and the design of protective spectacles

This is a most valuable book to electroencephalographers and epileptologists With its clarity and balance its many case histories 60 illustrations of good quality and a well chosen and useful bibliography it is however of real interest and value to all who are concerned with clinical aspects of brain function

Karl Magnus Herrlin

H C Shirkey (ed) *Pediatric therapy* 5th ed 1405 pp illus The C V Mosby Company St Louis 1975 US $41 50

Pediatric therapy is now out in its 5th edition the 4th one was published only 3 years ago and right from the beginning one can say that there are no remarkable new changes in this edition The size of the book is about the same 1405 pages written by altogether 117 contributors There are 490 modern and good illustrations

Since the 1st edition back in 1964 Pediatric therapy has followed roughly the same course and covers roughly the same fields of pediatric therapy Compared with the latest edition in 1972 there are exactly the same chapters in this new edition although some of the chapters have been brushed up in order to keep pace with modern development in pediatric therapy

By tradition Pediatric therapy has had strong chapters concerning drug treatment adverse drug reactions general therapy and infectious diseases—these chapters are still strong and up to date Other chapters may be called weak ones and those are still weak for instance the chapter concerning the newborn infant pediatric psychiatry allergic disorders and pediatric neurology The presentation in these chapters shows that it today no longer is appropriate to deal with all fields of pediatrics in one volume It is almost impossible for instance to cover modern aspects of treatment in pediatric neurology in 18 pages and the newborn infant in 20 pages!

Editors also of classical textbooks such as Pediatric therapy have to accept that modern development is so rapid and complicated that the time has passed when one could cover all aspects in one textbook However with this general critical remark in mind Pediatric therapy is still an extremely useful and valuable textbook especially concerning drug therapy in pediatrics!

Ove Petersson

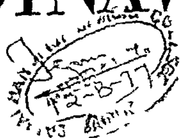
ERRATUM

In the article by K Scharer et al Dialysis and renal transplantation of children in Europe 1974 vol 65 pp 657–662 1976 is a printing error On p 662 left column the second line should read

ological age in 70% of all children and above

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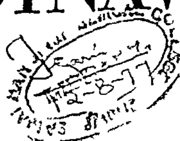
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Acta Pædiatr Scand 66 1–6 1977

PAEDIATRICS TODAY AND TOMORROW[1]

BO VAHLQUIST[2]

Paediatrics today and tomorrow—this is rather a question of futurology Of this it has been said that the only certainty is that the future arrives more quickly than we have imagined and that it is different from what we have assumed The distance in time up to the year 2000 is no longer than that back to 1952—and for many of us that does not seem such a long time ago

Our society is undergoing a fundamental process of change Man as a biological individual is not affected by this but his development and adaptation to the constantly changing environment are being considerably influenced No prognoses can be made about tomorrow s paediatrics without first considering our expectations concerning future alterations in the life milieu of children and adults and possible further advances in medical research

During the past century the general standard of living in our Scandinavian countries has been raised tremendously *At the same time* the change over from an agricultural to an industrial community has basically altered our *ancient traditions* The extended family with numerous offspring has given way to the nuclear family with limited offspring and poor contact with relatives A large proportion of women have obtained work away from the home As a result the need for daily institutional care of children of preschool age has greatly increased There is reason to fear that tomorrow s parents may tend to consider even the care of healthy children as primarily the concern of the community with a reduction of their own responsibility and greater insecurity on the part of the children as a result The development of mass media especially television is progressively replacing active contact with play sports and nature by standardized passive consumption of filmed material

Advances in medical research such as sulphonamides antibiotics and vaccines have favoured paediatrics probably more than any other of the traditional medical disciplines It seems as if the most beautiful fruits have already been harvested—as long as our hopes of effective measures against the constantly prevalent common cold are not realised

Let us now consider for a moment some facts concerning the *population development* which so especially concerns the paediatrician The birth rate has declined greatly in all Scandinavian countries with immediate effects on the size of the child population In the mid *1940s 135 000* children were born annually in Sweden while in 1975 this figure was down to 103 000 In the Scandinavian countries today *children* under 15 years of age constitute no more than about 1/5 of the total population A stabilisation of this development might possibly be expected in the years to come It seems that both contraception and legal abortions have now reached some degree of balance One advantage of these radical measures is that the majority of the children now entering our part of the world are wanted children

The infant mortality in the Scandinavian

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[1] Inaugural address delivered on June 30 1976 on the occasion of the 18th Nordic Paediatric Congress Århus Denmark

[2] Em Professor of Paediatrics Department of Paediatrics University Hospital Uppsala Sweden

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countries lies at an unprecedentedly low level—both Finland and Sweden now report figures below 10/1 000 liveborns This means that whole nations now approach the low level that 50 years ago could only be claimed by the royal families The mortality after the first year of life has shown a similar trend The time is past when small coffins were frequently seen being carried for burial at our church yards When on the other hand a child does die the loss is perhaps even harder to bear

Underlying the previously high mortality in infancy lay two major disease complexes—serious infectious diseases and severe malnutrition The latter has long since been eliminated and the former is well on its way to this goal

While many of the old diseases have been completely eradicated (polio, diphtheria rheumatic fever tuberculosis etc) or greatly reduced (pneumonia whooping cough scarlet fever meningitis sepsis etc) others have gained relative importance Robert J Haggerty speaks of the new morbidity This concept includes both physical and mental problems In the former category we find for example the sudden unexpected death in infancy childhood accidents the battered child syndrome the allergic diseases and the comprehensive care of the handicapped child and in the latter behavioural disturbances and adolescent and social problems Paediatricians active in outpatient and ambulatory care in particular will be concentrating their attention increasingly on the new morbidity in the next decades

In recent years there have been lively debates in all the Scandinavian countries on the *organisation of medical care* with special regard to primary care (lowest acceptable level of care) It is important that this should as far as possible be the responsibility of the general practitioner the specialist being reserved only for selected patients But the paediatric specialist in outpatient care is also a general practitioner—for children! Parents are increasingly expressing a wish to consult a paediatrician when their child is ill The total volume of the child medical care need is extensive—it is estimated that about 1/4 of all outpatient cases are children In addition there is a need for extensive engagement in all sorts of child health activities

The Swedish Board of Health and Welfare have now devised a plan whereby special district paediatricians are to be established It is intended that with the expansion of primary care and as more paediatricians become available such positions shall be created throughout the country It will be several decades however before this plan can be fully realised The present number of paediatricians has to be more than doubled

I have spoken of paediatricians in ambulatory care as general practitioners for children In hospital care, also paediatricians with a good all round training are required But here there is an increasing demand especially in the large university hospitals for paediatricians with wide experience in special fields such as neurology habilitation cardiology allergology etc For a long time such specialisation took place on the basis of personal interest but more recently in many places permanent specialist positions have been established Continued development in this direction is to be expected Space does not allow a detailed discussion here but I should like to touch upon one question namely *perinatal care* The valley of the shadow of birth is a sombre expression As little as 25 years ago 30–40/1 000 children in Sweden died in the perinatal period i e in the final stage of pregnancy during the delivery itself or in the first week of life This mortality rate was as high as during the entire subsequent period up to the age of 30 years It is one of the most beautiful examples of the results of improved medical efforts and especially of cooperation over the borders between obstetricians anaesthesiologists and paediatricians that the perinatal mortality has now fallen to 13/1 000 In one hospital Danderyd near Stockholm where this question has received special at

tention a figure of 8/1 000 has been attained for three consecutive years mainly by simple measures At the same time investigations in Gothenburg as well as in other parts of Scandinavia have shown that among the children whose lives have been saved in this way there has been no increase in the frequency of handicaps There is no doubt that augmented efforts in perinatal care will be one of the characteristics of the paediatrics of tomorrow

In this connection I will make a few brief comments on the need of *hospital beds for children* If we estimate that to an increasing extent children will receive care at paediatric clinics regardless of the nature of their basic disease the old rule of thumb of 30 beds per 100 000 population should not become much lower

More important than the number of beds is the way in which they are utilized and the quality of the general care of the children There have been considerable improvements in recent years—parents are encouraged to be near their child systematic play therapy and school lessons are favoured and painful and frightening procedures are avoided as far as possible But even today not all paediatric clinics live up to reasonable demands in these respects and certain alterations in the medical care milieu resulting from structural changes in society are of a directly negative nature Shortened working hours and increasingly quick turn-over of staff result in a reduction of the children s sense of security

In paediatric care both inpatient and ambulatory *behavioural disturbances* are playing a progressively larger role There is clearly not only a relative but also an absolute increase in this respect Certain features in the development of our society provoke such disturbances at the same time as the uncertainty of the parents lowers their own and their child s tolerance limit Often it is a case of a simple problem where enlightenment on normal biological and age variations will be sufficient "treatment In other cases advice and guidance in the field of minor child psychiatry is needed More and more frequently behavioural disturbances prove to have arisen from social problems Our welfare state seems rather to constitute a breeding ground for such disturbances and it seems likely that this will be increasingly reflected in tomorrow s paediatrics The need for intimate collaboration with various welfare agencies will then become even greater

Adolescence easily becomes a no man s land In several places Finland in particular special adolescent clinics have been organised following the pattern established in the USA The predominant problems are of a psychosomatic hypochondrial and emotional nature on the one hand and endocrinological and gynaecological—including contraception—on the other These clinics have been found to fill an important need and this type of activity will probably be extended in the future More attention than previously should also be paid in this connection to invoking sound dietary habits in young people

Preventive health care It is clear that in childhood in particular preventive health care has brought great benefits In Sweden a government supported activity in this field was launched in 1938 It rapidly gained momentum and today well covers the population Health care begins at birth or rather before birth and continues at child health centres day nurseries and schools The linking key figure is often the district nurse who has the best contact with the family and represents continuity The paediatrician cannot afford the same amount of time for each patient but his or her role in providing support consultation and instruction cannot be overestimated

There is much discussion today about the *special health screening of 4 year olds* In Sweden this began in 1967 and it is now being carried out to varying extents and by varying methods in all Scandinavian countries It may and should be regarded as a link in the general preventive health care of preschool children It is of value not only because of the new sometimes very important observations that

countries lies at an unprecedentedly low level—both Finland and Sweden now report figures below 10/1 000 liveborns This means that whole nations now approach the low level that 50 years ago could only be claimed by the royal families The mortality after the first year of life has shown a similar trend The time is past when small coffins were frequently seen being carried for burial at our church yards When on the other hand a child does die the loss is perhaps even harder to bear

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I have spoken of paediatricians in ambulatory care as general practitioners for children In hospital care also paediatricians with a good all round training are required But here there is an increasing demand especially in the large university hospitals for paediatricians with wide experience in special fields such as neurology habilitation cardiology allergology etc For a long time such specialisation took place on the basis of personal interest but more recently in many places permanent specialist positions have been established Continued development in this direction is to be expected Space does not allow a detailed discussion here but I should like to touch upon one question namely *perinatal care* The valley of the shadow of birth is a sombre expression As little as 25 years ago 30–40/1 000 children in Sweden died in the perinatal period i e in the final stage of pregnancy during the delivery itself or in the first week of life This mortality rate was as high as during the entire subsequent period up to the age of 30 years It is one of the most beautiful examples of the results of improved medical efforts and especially of cooperation over the borders between obstetricians anaesthesiologists and paediatricians that the perinatal mortality has now fallen to 13/1 000 In one hospital Danderyd near Stockholm where this question has received special at

tention a figure of 8/1000 has been attained for three consecutive years mainly by simple measures At the same time investigations in Gothenburg as well as in other parts of Scandinavia have shown that among the children whose lives have been saved in this way there has been no increase in the frequency of handicaps There is no doubt that augmented efforts in perinatal care will be one of the characteristics of the paediatrics of tomorrow

In this connection I will make a few brief comments on the need of *hospital beds for children* If we estimate that to an increasing extent children will receive care at paediatric clinics regardless of the nature of their basic disease the old rule of thumb of 30 beds per 100000 population should not become much lower

More important than the number of beds is the way in which they are utilized and the quality of the general care of the children There have been considerable improvements in recent years—parents are encouraged to be near their child systematic play therapy and school lessons are favoured and painful and frightening procedures are avoided as far as possible But even today not all paediatric clinics live up to reasonable demands in these respects and certain alterations in the medical care milieu resulting from structural changes in society are of a directly negative nature Shortened working hours and increasingly quick turn-over of staff result in a reduction of the children s sense of security

In paediatric care both inpatient and ambulatory *behavioural disturbances* are playing a progressively larger role There is clearly not only a relative but also an absolute increase in this respect Certain features in the development of our society provoke such disturbances at the same time as the uncertainty of the parents lowers their own and their child s tolerance limit Often it is a case of a simple problem where enlightenment on normal biological and age variations will be sufficient treatment In other cases advice and guidance in the field of minor child psychiatry is needed More and more frequently behavioural disturbances prove to have arisen from social problems Our welfare state seems rather to constitute a breeding ground for such disturbances and it seems likely that this will be increasingly reflected in tomorrow s paediatrics The need for intimate collaboration with various welfare agencies will then become even greater

Adolescence easily becomes a no man s land In several places Finland in particular special adolescent clinics have been organised following the pattern established in the USA The predominant problems are of a psychosomatic hypochondrial and emotional nature on the one hand and endocrinological and gynaecological—including contraception—on the other These clinics have been found to fill an important need and this type of activity will probably be extended in the future More attention than previously should also be paid in this connection to invoking sound dietary habits in young people

Preventive health care It is clear that in childhood in particular preventive health care has brought great benefits In Sweden a government supported activity in this field was launched in 1938 It rapidly gained momentum and today well covers the population Health care begins at birth or rather before birth and continues at child health centres day nurseries and schools The linking key figure is often the district nurse who has the best contact with the family and represents continuity The paediatrician cannot afford the same amount of time for each patient but his or her role in providing support consultation and instruction cannot be overestimated

There is much discussion today about the *special health screening of 4 year olds* In Sweden this began in 1967 and it is now being carried out to varying extents and by varying methods in all Scandinavian countries It may and should be regarded as a link in the general preventive health care of preschool children It is of value not only because of the new sometimes very important observations that

countries lies at an unprecedentedly low level—both Finland and Sweden now report *figures below 10/1 000 liveborns* This means that whole nations now approach the low level that 50 years ago could only be claimed by the royal families The mortality after the first year of life has shown a similar trend The time is past when small coffins were frequently seen being carried for burial at our church yards When on the other hand a child does die the loss is perhaps even harder to bear

Underlying the previously high mortality in infancy lay two major disease complexes—serious infectious diseases and severe malnu trition The latter has long since been elimi nated and the former is well on its way to this goal

While many of the old diseases have been *completely eradicated (polio diphtheria* rheumatic fever tuberculosis etc) or greatly reduced (pneumonia whooping cough scarlet fever meningitis sepsis etc) others have gained relative importance Robert J Hagger ty speaks of the new morbidity This con cept includes both physical and mental prob lems *In the former category we find* for ex ample the sudden unexpected death in in fancy childhood accidents the battered child *syndrome the allergic diseases and the com* prehensive care of the handicapped child and in the latter behavioural disturbances and adolescent and social problems Paediatricians *active in outpatient and ambulatory care in* particular will be concentrating their attention increasingly on the new morbidity in the next decades

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cigarett smoking sexual promiscuity over nutrition wrong dietary habits etc are enormous Against this background the efforts to influence the underlying attitudes seem fumbling and uncertain It is difficult to believe that the life style aberrations can be allowed to continue These problems certainly deserve increasing involvement on the part of the medical profession

Ethical problems Hitherto these have been discussed mostly in relation to clinical research In the future they will certainly become more topical and more penetrating also in practical health care including paediatrics Economic and personnel limitations may force decisions concerning priorities that will prevent patients from benefiting from new advances in medical research Questions on how far the concentration of resources on the incurable should be carried may become a painful reality Genetic counselling may in its implementation involve intricate decisions

In this connection one particular problem of ethics may be mentioned—that of the doctor s liability In the USA we are witnessing today a wave of claims for damages against doctors for malpractice—true or supposed Our experiences of this in Scandinavia hitherto are only the beginning

Paediatricians and the Third World Social responsibility today does not end at national borders We live in a rapidly shrinking world Satellite pictures reach our eyes almost at the same moment as the dramatic events that they depict are taking place in Beirut Johannesburg or Luanda The victims of starvation in Ethiopia Sahel and Bangladesh gaze at us with dark and desperate eyes What do we do for our brothers and sisters in the Third World? The paper mills grind at large conferences but the practical result is usually desperately insufficient to diminish the constant silent need among hundreds of millions of children in the developing countries It is hardly likely that their lot will be improved appreciably until radical political changes have been made Regimes are needed that will become unequivocably involved for the benefit of the enormous groups of indigent people and which will place the needs of mothers and children not last but first

In relation to the world as a whole the Scandinavian countries are small both population wise and economically but their voices are nevertheless relatively powerful It is important that their messages be based on firm ground established also by the collaboration of the medical profession Many Scandinavian colleagues especially paediatricians have taken part and are doing so today in development assistance in the Third World Their number could be greater however—we could afford this

During the 15 years in which I myself have been engaged in related problems the aim and form of development assistance has changed in many respects In 1960 40 of Africa s 49 states were under Colonial rule whereas today only three remain The numerous new independent states often with Marxist ideals are filled with a strong will to shape their own development and their own destiny Cooperation from industrialized countries is still relevant in the field of health care but the roles of the expatriate resource persons have changed and the demands for good previous knowledge of conditions prevailing in the Third World and willingness to accept national priorities in health care have increased

As part of a systematic training for work in Third World countries 10-week training courses for doctors and nurses combined have been run in Sweden since 1973 under the auspices of the National Board of Health and Welfare in cooperation with the Swedish International Development Authority (SIDA) and with collaboration between university institutions in Uppsala and Stockholm Altogether more than 200 doctors and registered nurses most of them already contracted for work in developing countries have now received their diploma To a limited extent the courses which are held twice a year have also been open to participants from the neighbouring

are made concerning impaired vision and hearing etc but also because it has aroused interest in new test methods, promoted group activity and initiated the establishment of new posts The chief officer of child health care at county level has become the central figure linking together the entire chain of preventive health care measures at preschool ages

The district paediatricians in Sweden often use one third (or more) of their working time for preventive care Benefits may be assumed not only in the form of improved health in childhood but also far later in adult years

School health care at least in Sweden has not yet achieved an ideal form This autumn we foresee a new parliamentary committee report in this field probably without major proposals for change Recommendations are expected for renouncing the strict health care nature of this service to allow the addition of simple medical treatment

The school doctor has much to gain by following the paediatric model for contacts with the pupils and their parents But he or she also needs to be able to collaborate—for example with teachers psychologists and social workers In Sweden there has been much debate about SIA—a study on the school routine This aims at a more integrated school day team work and freer availability of resources and in general a more open school The health care of school children will certainly be affected but little of this has been mentioned in the committee reports

The paediatrician as a group worker The development has long moved in the direction of increasing specialisation not only in medicine but in all occupational fields It is unlikely that this trend will reverse and it therefore has to be balanced by group work One has to learn to understand and speak the language of others while at the same time preserving one s own special ability For the paediatrician this means that he must be as well versed as ever in the basic knowledge of the growth and development of the child and its mode of reaction to different environmental factors or in his detailed knowledge of all the major childhood diseases—including the psychosomatic conditions and minor child psychiatry In these areas the paediatrician cannot be replaced And group work is not only a question of willingness to cooperate and freedom from prestige—to be successful it requires solid knowledge on the part of its participants

The paediatrician s working day For the majority of paediatricians—especially those in district care—the work is not particularly exciting or glamorous perhaps not even interesting in the sense that it gives the opportunity to subtle diagnoses or dramatic cures Rather it is a strenuous sometimes monotonous job of a service type where the reward lies in the knowledge that one has close contact with the patient and his family and is at hand in times of trouble with encouragement and good advice With the right selection of candidates to medicine as a profession and especially to paediatrics this should be satisfaction enough

The paediatrician as a custodian over children s interests In American paediatric circles the paediatrician has sometimes been referred to as the ombudsman of children Pleasingly enough in our Scandinavian countries there has always been a strong social interest among paediatricians They have not been satisfied with merely treating a child s illness but have gone one step further In this way they have exerted considerable influence in preventive and social paediatrics There would seem reason to ask however in these times of radical change if the voice of the paediatrician should not be heard even louder in current debates

The life style crisis In the criticism against modern medicine that flares up from time to time and that may be expected to increase in strength in coming years it has been pointed out that the severe health problems of the wealthy nations can only be solved by a change in the general life style The harmful effects on the individual and community of traffic accidents alcohol and misuses of drugs

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Acta Pædiatr Scand 66 7–15 1977

HEREDITARY CHOLESTASIS COMBINED WITH PERIPHERAL PULMONARY STENOSIS AND OTHER ANOMALIES

N T HENRIKSEN F LANGMARK S J SØRLAND O FAUSA
S LANDAAS and Ø AAGENAES

From the Department of Paediatrics Department of Pathology Medical Department A and Department of Clinical Chemistry University Hospital Rikshospitalet Oslo Norway

ABSTRACT Henriksen N T Langmark F Sørland S J Fausa O Landaas S and Aagenaes O (Department of Paediatrics Department of Pathology Medical Department A and Department of Clinical Chemistry University Hospital Rikshospitalet Oslo Norway) Hereditary cholestasic combined with peripheral pulmonary stenosi and other anomalies. Acta Paediatr Scand 66 7 1977.—A syndrome consisting of chronic intrahepatic cholestasis with retention of bile acids but with normalization of bile pigment excretion and blood lipids peripheral pulmonary stenosis vertebral anomalies and a characteristic facies is described in six patients including a father and his daughter An autosomal dominant mode of inheritance is suggested

KEY WORDS Intrahepatic cholestasis retention of bile acids peripheral pulmonary stenosis vertebral anomalies characteristic facies autosomal inheritance

In 1970 Alagille & Thomassin (1) presented 25 children with chronic intrahepatic cholestasis with constant or intermittent jaundice severe itching and hepatomegaly A characteristic facies was noted in some of them and in some a stenotic systolic cardiac murmur was heard In many of the patients there was found a partial absence of the intrahepatic interlobular bile ducts Later Watson & Miller (16) described a syndrome with the following salient features 1) congenital hypoplasia with stenosis of the main pulmonary arteries 2) neonatal liver disease with cholestasis and 3) various minor congenital anomalies including an odd facies Recently Alagille et al (2) reported that intrahepatic hypoplasia of the bile ducts was the main hepatic morhpological feature of the syndrome Other striking features were characteristic facies vertebral malformations retarded physical mental and sexual development and cardiac murmur

At the Paediatric Department Rikshospitalet National Hospital of Norway Oslo we have studied this syndrome in 6 patients including a father and his daughter

LABORATORY METHODS

The enzyme concentrations in serum have been determined over a period of 12 years and different methods have therefore been applied The following method numbers correspond to the index numbers in Table 3 where the reference intervals for adults are also shown for each method Alkaline phosphatase alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) (methods 2 4 and 6 respectively) are today measured on the basis of the methods recommended by the Scandinavian Committee on Enzymes Alkaline phosphatase was earlier determined (method 1) by the method applied by Bessey & Lowry (?5?) and the transaminases (methods 3 and 5) by the method of Reitman & Frankel (37?) Ornithine carbamyl transferase (OCT) (methods 7 and 8) has been measured by two different modifications of the isotope method of Reichard (37?)

The serum levels of cholesterol were determined photometrically by the Liebermann Burchard reaction and triglycerides by the method of Laurell

Scandinavian countries We hope that this Scandinavian collaboration will develop and become consolidated

Let me end this short expose with two quotations one by Rene Dubos, the other by John Apley

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The most important question mankind can ask itself is When our children grow up what sort of adults and parents will they be? To find answers and to help them and their children in turn we must study the psychosocial milieu And we should constantly remind ourselves that *all childhood is a critical period*

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Table 3 *Biochemical parameters*

The index numbers for the enzymes refer to the methods applied (see text)

Patients	Alk phos μ/l		ASAT μ/l		ALAT μ/l		OCT μ/l		Cholesterol mg/100 ml		Triglycerides mg/100 ml		Bilirubin mg/100 ml		Bile acids (serum) mmol/l
	Infancy 1)	Recent 2)	Infancy 3)	Recent 4)	Infancy 5)	Recent 6)	Infancy 7)	Recent 8)	Infancy	Recent	Infancy	Recent	Infancy	Recent	Recent
T R	–	1 088	–	76	–	94	–	286	–	240	–	170	–	1 1	16 2
J H	–	840	–	54	–	70	–	370	–	245	–	70	–	1 0	20 9
T O	25	1 300	235	104	140	102	–	816	–	165	–	80	5 5	1 2	10 0
C H	38	405	100	112	127	134	–	1 270	256	215	–	85	3 8	0 5	4 1
A H	70	2 000	38	210	24	110	570	–	–	335	–	310	7 0	3 5	44 6
A K R	97	1 200	86	128	78	114	1 310	912	–	250	–	90	5 6	0 6	58 9
Reference interval for adults	10–45	55–195	5–20	13–38	2–17	10–40	0–350	0–70							

ALAT and ASAT) except in A H when she was a neonate

The cholesterol concentration early in infancy was determined in only one patient (C H) A moderate increase was found Later in childhood it was even more increased but now there is no hyperlipemia Similarly the two youngest patients (3 and 3½ years old) have hyperlipemia while the other older patients have normal lipid concentration in relation to age

The serum concentrations of total bile acids (Table 3) are increased in all the patients except one and in some of them the values are very high Bile acid separation has shown no unusual bile acids (Prof Eyssen)

Liver biopsies Table 4 shows that altogether 12 biopsies were taken from the six patients The main morphological criterion for the diagnosis is lack or paucity of bile ducts in the portal areas One patient had a completely normal liver biopsy (needle) In the other patients the number of portal areas containing bile ducts varied from 0 to 50% of the total Bile stasis with bile plugs in the bile capillaries and accumulation of bile pigment in

Table 4 *Liver biopsy studies*

N=needle S=surgical

Patient	Age of the time of examin	Biopsy	No of portal areas	No of bile ducts	Bile stasis	Inflam changes	Hep cell degen	Fibrosis
T R	30 y	N	11	3	–	–	–	–
J H	10 y	N	2	2	–	–	–	–
T O	2 m	S	21	9	+	+	+	–
	11 y	N	11	3	–	–	–	–
C H	1½ y	S	21	0	–	–	–	–
	5 y	N	8	2	–	–	–	–
A H	1 m	N	1	0	+	–	–	–
	3 m	N	10	0	+	+	+	–
	7 m	N	3	0	+	+	+	–
	12 m	N	3	0	+	+	+	+
	25 m	N	3	0	(+)	+	(+)	++
A K R	1 m	S	30	15	+	+	+	–

Table 1 *The clinical course of the liver disease*

Where not at present is noted the information from infancy and (early) childhood is not available

Patient	Sex	Age (y)	Birth weight (g)	Birth length (cm)	Jaundice	Pruritus	Hepatomegaly	Steatorrhea
T R	M	30			In infancy	In childhood	Not at present	Not at present
J H	M	12	3 140	51	Never	Persists	Never	Not at present
T O	M	11	3 080	48	In infancy	Persists	In infancy	In infancy
C H	M		2 310	45	In infancy	Some years	In infancy	In infancy
A H	F	3½	2 020	46	Persists	Persists	Persists	Persists
A K R	F	3	2 430	47	In infancy	Persists	Persists	In infancy

The bile acids shown in Table 3 were determined by the method described by Fausa (10) the upper normal limit is considered to be 6 mmol/l

Gas liquid chromatography of bile acids was performed by Prof H Eyssen Brussels

The endocrinologic analyses were carried out by the Hormone and Isotope Laboratory Aker Hospital Oslo

RESULTS

Table 1 and 2 show the clinical course in the patients They were small for date at birth with an average birth length of 47 cm All the patients except one of the boys (J H) have a history of neonatal cholestasis Exploratory laparotomy was performed in three and they have normal extrahepatic bile ducts The liver was enlarged in all the patients except in the nonicteric boy (J H)

Jaundice was of short duration in most of the patients while pruritus continued for a long time and still persists in two of the boys 11 and 12 years old Following the disappearance of jaundice, the liver size became normal Steatorrhea was constant during the severe cholestatic period in infancy but then gradually improved Only one girl (A H) still has steatorrhea Table 3 shows the biochemical changes in our patients The bilirubin concentration was increased during the neonatal period (and also later in infancy) but the values are now below the upper normal limit in all the patients except A H With regard to the liver enzymes OCT is increased in all the patients The serum concentrations of alkaline phosphatase is normally much higher in children and especially in infants than in adults and all the neonatal values are within the normal range for this age group However the alkaline phosphatase levels are at present increased The concentrations of the two transaminases are increased in relation to age in all patients (2–4 times upper normal limit for both

Table 2 *Characteristics Physical mental and sexual development*

N=normal R=retarded (R)=transiently retarded NE=not examined

Patient	Age (y)	Charact facies	Vertebral anomaly	Endocrine abnorm	Development: Growth	Mental	Sexual
T R	30	+	+	NE	N(?)	N	N
J H	12	+	+	0	(R)	N	N
T O	11	+	+	0	N	N	N
C H	10	+	+	0	(R)	N	N
A H	3½	+	0	+	R	R	
A K R	3	+	+	0	N	N	

The following tests have been done T4 PBI daily urinary excretion of 17 ketogenic steroids cortisol in serum FSH LH TSH and HGH In patients T O and C H testosterone in serum was measured before and after stimulation with choriongonadotropin

Table 3 *Biochemical parameters*

The index numbers for the enzymes refer to the methods applied (see text)

Patients	Alk phos µ/l In fancy 1)	Alk phos µ/l Re cent 2)	ASAT µ/l In fancy 3)	ASAT µ/l Re cent 4)	ALAT µ/l In fancy 5)	ALAT µ/l Re cent 6)	OCT µ/l In fancy 7)	OCT µ/l Re cent 8)	Choles terol mg/100 ml In fancy	Choles terol mg/100 ml Re cent	Triglyce rides mg/100 ml In fancy	Triglyce rides mg/100 ml Re cent	Bili rubin mg/100 ml In fancy	Bili rubin mg/100 ml Re cent	Bile acids (serum) mmol/l Recent
T R	–	1 088	–	76	–	94	–	286	–	240	–	170	–	1 1	16 2
J H	–	840	–	54	–	70	–	370	–	245	–	70	–	1 0	20 9
T O	25	1 300	235	104	140	102	–	816	–	165	–	80	5 5	1 2	10 0
C H	38	405	100	112	127	134	–	1 270	256	215	–	85	3 8	0 5	4 1
A H	70	2 000	38	210	24	110	570	–	–	335	–	310	7 0	3 5	44 6
A K R	97	1 200	86	128	78	114	1 310	912	–	250	–	90	5 6	0 6	58 9
Reference interval for adults	*10–45*	*55–195*	*5–20*	*13–38*	*2–17*	*10–40*	*0–350*	*0–70*							

ALAT and ASAT) except in A H when she was a neonate

The cholesterol concentration early in in fancy was determined in only one patient (C H) A moderate increase was found Later in childhood it was even more increased but now there is no hyperlipemia Similarly the two youngest patients (3 and 3½ years old) have hyperlipemia while the other older pa tients have normal lipid concentration in rela tion to age

The serum concentrations of total bile acids (Table 3) are increased in all the patients ex cept one and in some of them the values are very high Bile acid separation has shown no unusual bile acids (Prof Eyssen)

Liver biopsies Table 4 shows that alto gether 12 biopsies were taken from the six pa tients The main morphological criterion for the diagnosis is lack or paucity of bile ducts in the portal areas One patient had a com pletely normal liver biopsy (needle) In the other patients the number of portal areas containing bile ducts varied from 0 to 50% of the total Bile stasis with bile plugs in the bile capillaries and accumulation of bile pigment in

Table 4 *Liver biopsy studies*

N=needle S=surgical

Patient	Age of the time of examin	Biopsy	No of portal areas	No of bile ducts	Bile stasis	Inflam changes	Hep cell degen	Fibrosis
T R	30 y	N	11	3	–	–	–	–
J H	10 y	N	2	2	–	–	–	–
T O	2 m	S	21	9	+	+	+	–
	11 y	N	11	3	–	–	–	–
C H	1½ y	S	21	0	–	–	–	–
	5 y	N	8	2	–	–	–	–
A H	1 m	N	1	0	+	–	–	–
	3 m	N	10	0	+	+	+	–
	7 m	N	3	0	+	+	+	–
	12 m	N	3	0	+	+	+	+
	25 m	N	3	0	(+)	+	(+)	++
A K R	1 m	S	30	15	+	+	+	–

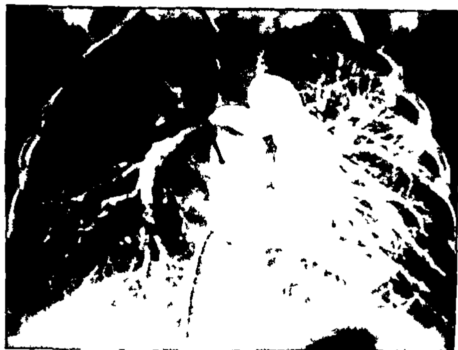


Fig 1 Stenosis of the right pulmonary artery (arrow) in one of the patients (A K R)

the hepatocytes was a prominent feature in biopsies taken during the first 12 months of life confirming the clinical and biochemical findings In these biopsies a variable degree of inflammatory reaction with granulocytes and lymphocytes in the portal areas was also found frequently together with swollen and granulated hepatocytes In older patients no pathological liver findings were seen at light microscopy except lack of ducts in a variable number of portas areas In the case with the most severe clinical history (A H) the liver also revealed the most severe changes with increasing fibrosis from the second year of life None of the biopsies showed giant cell transformation

Cardiac findings All the patients have a systolic cardiac murmur and the results of the cardiac examinations are presented in Table 5 The murmur is shown by catheterisation to be caused by stenoses in the great pulmonary arteries There were no other cardiac anomalies The stenoses were located on different site in the peripheral pulmonary tree with the gradient over the stenoses ranging from 10 to 35 mm Fig 1 shows stenosis on the right side in the main pulmonary vessel in one of the patients (A K R) None of the patients has cardiac symptoms

Facies A characteristic facies is present in all (Figs 2 3 and 4) There are slight hypertelorism the eyes are deeply set the chin

Table 5 *Cardiac findings*
PPS=peripheral pulmonary stenosis

Patient	Clinical		Heart catherisation findings		
	Heart murmur	Heart symptom	Pathology	Location	Gradient (mmHg)
T R	+	0	PPS	Supravalvular	20
J H	+	0	PPS	Left side	15
T O	+	0	No catheter study		
C H	+	0	PPS	Main trunk	10
A H	+	0	PPS	Both sides	25
A K R	+	0	PPS	Both sides	35

Clinical diagnosis PPS



Fig 2 One of the patients (A H) in infancy



Fig 4 One of the patients (T O) 11 years old



Fig 3 Another patient (C H) in infancy

bones are wide apart and the forehead is broad The profile is flat

Vertebrae Most of the patients have verte bral anomalies (Table 2) and these are of dif ferent kinds hemivertebra cleft in the verte bral body unsegmented body wedgeshaped body and in one patient spina bifida oculta

Growth Three children were growth re tarded two of them transiently while one still is (Table 2) The growth curve of one of them (C H) (Fig 5) is at the 2 5 percentile at 2 years and 50 percentile at 10 years

Mental development Only one child is re tarded (Table 2)

Endocrinological examinations The hor mone analyses including T4 PBI daily excre tion of 17 ketogenic steroids cortisol in se rum FSH LH TSH and HGH were all nor mal except extremely high values for the growth hormone in the severely retarded girl (A H) (Table 2) The size of the testes in our three prepubertal boys is normal Testoster

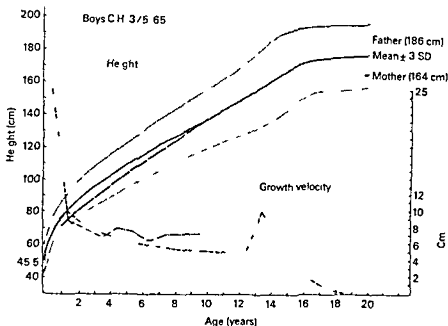


Fig 5 Growth curve and growth velocity curve in one of the pre pubertal boys (C H)

one measured before and after choriongonado tropin stimulation showed normal values (performed in only two of the three boys)

Family observations (Fig 6) The father and a younger sister of one patient (J H) have a systolic murmur probably caused by periph eral pulmonary stenosis The father of one of the others (A H) has a similar cardiac mur mur The five children have altogether five siblings Aside from the father included in our study there is no evidence of liver disease in the family members

Other examinations Examinations for an infectious etiology of the liver disease have been negative Alfa 1 antitrypsin levels are within normal limits Chromosome analyses performed in five of the patients are normal

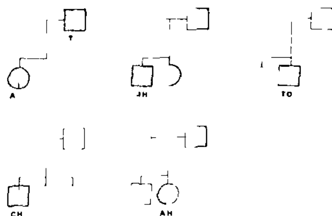


Fig 6 The parents and their children in the affected families Vertical lines=cholestasis Horizontal lines= peripheral pulmonary stenosis

DISCUSSION

The liver disease The clinical picture in our patients is dominated by the liver disease most often presenting as a neonatal cholesta sis The most prominent symptom is itching persisting for many years after the jaundice disappeared and the bilirubin became normal One patient was not jaundiced at all Itching in liver or biliary disease is thought to be caused by retention of bile salts Most often they are retained together with the bile pig ments because of obstruction in the common excretion pathways resulting in the well known combination of jaundice and pruritus It is however a well established fact that the excretory mechanisms for bile acids are not the same as for bilirubin Norman & Strandvik (14) found disturbed excretion of bile acids and elevated serum transaminases for up to more than a year after the disappearance of jaun dice in a number of patients who had intra hepatic cholestasis in infancy In a patient with the disorder known as benign recurrent intrahepatic cholestasis (3) the serum bile acids were found to be considerably increased without an initial increase of bilirubin The

laboratory analyses (Table 3) show the dis sociation between the secretion of bile acids and of bilirubin in our patients The levels of serum bile acids are variously elevated from borderline to very high values while bilirubin levels have become normal in all patients ex cept one

There seems to exist only partial correla tion between the serum bile acid levels and the itching in our patients However as stated by Luders (13) it has not been proven that the total bile acids or any bile acid at all are di rectly responsible for the itching Perhaps an unknown substance is retained together with the bile acids On the other hand Luders emphasizes the importance of disturbed bile acid metabolism in the pathogenesis of liver damage with cholestasis

Disturbances of the bile acid metabolism have been shown (9 11) to be connected with a familial form of childhood cholestasis with hypoplasia of the intrahepatic bile ductules It was found high amounts of trihydroxyco prostanic acid in duodenal fluid as a result of defective conversion of this natural precursor to cholic acid in the liver cells In the patients of Eyssen et al (9) the metabolic acid defect was also connected with a number of congeni tal anomalies In our patients we have not found any unusual bile acid either in serum or in duodenal fluid which could point to a specific metabolic defect

The disappearance of steatorrhea suggests that the intestinal function of the bile acids is not severely affected and this suggestion was supported by the bile acid analyses in the dou denal fluid in two of the patients

Surprisingly enough we have not found that cholestyramine has had any convincing effect on the itching However the doses may not have been high enough or it may not have been taken in the prescribed way We have tried phenobarbital which is reported to have an effect on itching probably due to enzyme induction in the liver (15) There has been a clinical effect but no normalization of the pathological biochemical findings so far

The elevated levels of liver enzymes in serum are in accordance with a liver disorder involving both cell damage and a moderate cholestasis The normal values of alkaline phosphatase neonatally are probably due to the fact that isoenzymes originating from the bone will mask any elevation of liver enzymes if it is not very severe

Hyperlipemia has been present in the younger age group but not in the older pa tients

The pathological findings in the liver biop sies taken from the infants with this condition are not specific In only one of the twelve biopsies was the lack of bile ducts mentioned in the primary description but portal inflam mation frequently obscures the anatomical features (5) and makes the ducts hard to find particularly in needle biopsies in which portal areas may be insufficient in number for ade quate examination (six is considered a mini mum for being representative) Moreover there may be artefactual loss of whole portal areas for technical reasons

The diagnostic finding is paucity of bile ducts (4) Those which are present do not give any clue as to pathogenesis They look normal without signs of degeneration in flammation or obliteration but this may some times be difficult to judge in the icteric state because of the presence of general portal in flammation In older patients in the anicteric state the portal areas were completely normal with regard to number and size of portal ves sels No trace of bile duct disease was seen in areas devoid of ducts not even concentric fibrosis which is otherwise quite frequently seen in disuse atrophy of vessels where the scar tissue to some degree imitates the size and structure of the vanished vessel In many ways the patients with this syndrome do not seem to fit the concepts of Landing (12) on neonatal cholestasis and infantile obstructive cholangiopathy Studies on bile acid metab olism and transport in the liver may be more fundamental to the understanding of this dis ease

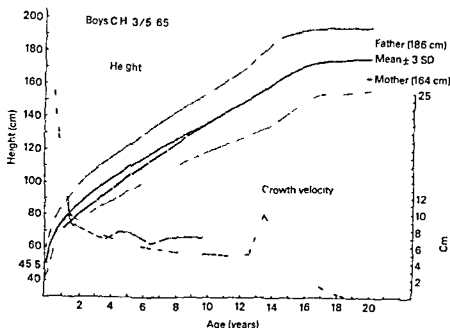


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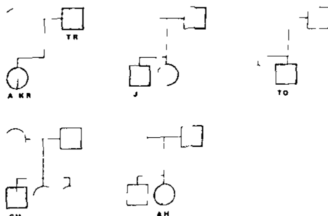


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The syndrome has been observed in 5 unrelated families living in or around Oslo Accordingly it does not seem to be rare and one should bear it in mind in patients with neonatal cholestasis and in patients with unexplained itching The syndrome is of great theoretical interest and may provide valuable information about metabolism and transport of bile acids in infants as well as adults

ACKNOWLEDGEMENT

We would like to thank Professor H Eyssen Brussels Belgium for performing bile acid agas liquid chromatography

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Submitted Dec 22 1975
Accepted Aug 16 1976

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Peripheral pulmonary stenosis This means a stenosis of the great pulmonary arteries pe ripheral to the valves Isolated it has hitherto been considered a rather rare defect It is thought to be nonprogressive (8) and there is a tendency to familial occurrence In Norway a family has been described (6) in which it was either isolated or associated with coarctation of the aorta It was shown that this condition was one of the few congenital heart diseases in which one single gene probably autosomal dominant was operative All our patients and probably two of the fathers and one sibling have isolated peripheral pulmonary stenosis

Facies Our patients as well as the others reported resemble each other like siblings This however, is not uncommon in congenital syndromes

Vertebral anomalies are frequent and of different types For the most part only one anomaly is present in each patient and it seems to be without clinical significance Ala gille et al (2) however related the growth retardation in their patients to vertebral anom alies mostly spina bifida defects

Growth retardation Two of our patients are in the lower range of normal height (2 5 to 10 percentile) and one is markedly retarded One patient had a cath up growth during a 10 year period (Fig 5) and during the same period his liver disease showed a definite improvement The markedly growth retarded patient (A H) is the one with the most severe liver disease She is our only patient without a vertebral anomaly One of the boys (J H) has during two years observation shown a small catch up growth from 2 5 towards 10 percentile These findings seem to indicate that the growth re tardation is directly related to the degree and duration of liver dysfunction and that there is no correlation between vertebral anomalies and growth retardation in our patients

Courtecuisse et al (7) reported extremely high concentrations of growth hormone in their growth retarded patients with hepatic ductular hypoplasia Our finding in the re tarded patient with a growth hormone level usually found in acromegalic patients is thus in accordance with the findings of the French workers The levels in our other patients were within normal range

Mentally our patients are normal except one of the girls (A H) The three schoolchildren manage their schoolwork well Alagille et al (2) found a moderate mental retardation (IQ 60–80) in 9 out of 15 patients This and other observations which differ from ours may be due to different methods of patient selection the French patients are all from a special pediatric liver unit and are probably more af fected by their liver disease than our patients

Gonadal function Clinical and hormonal examinations have not shown signs of hypo gonadism The fact that there among our pa tients is a father and his child also points against severe hypogonadism

Genetic aspect The family data are pre sented in Fig 6 which shows the mode of transmission of the disease Three of the fathers are probably affected by peripheral pulmonary stenosis and one also by the liver disease These observations taken with the 1 1 ratio of affected to unaffected children suggest an autosomal dominant genetic basis for the disorder This interpretation is in ac cordance with other observations on this syn drome (16)

CONCLUSION

Our 6 patients fit very well with the syndrome called arteriohepatic dysplasia by Watson & Miller (16) One main feature is a liver dis ease which usually starts as a neonatal chole stasis and after a time manifests itself as a chronic partial cholestasis with pruritus and increased levels of bile acids and liver en zymes but with normal bilirubin concentra tion We feel that the dysfunction primarily is caused by a defect in the metabolism or trans port of bile acids in the liver cells or bile cana liculi Long term observations indicate that the prognosis is quite good The other features of the syndrome are characteristic

Acta Pædiatr Scand 66 17–30 1977

SKIM MILK IN INFANT FEEDING

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ABSTRACT Fomon S J Filer L J Jr Ziegler E E Bergmann K E and Bergmann R L (Department of Pediatrics College of Medicine University of Iowa Iowa City Iowa 52242) Skim milk in infant feeding Acta Paediatr Scand 66 17 1977.—Ninety four infants were enrolled at 112 days of age in a study of food intake and growth and 88 were considered to have completed satisfactorily the planned 56 days of observation The infants lived at home Feedings consisted of a commercially available formula (Similac 67 kcal/100 ml) or a slightly modified skim milk (Formula 305 36 kcal/100 ml) and commercially prepared strained foods Energy intake and gain in weight were significantly greater by infants fed Similac than by those fed Formula 305 Gain in length was nearly identical in the two feeding groups During the 56 days of observation triceps and subscapular skin fold thicknesses changed little in infants fed Similac but decreased approximately 25% in infants fed Formula 305 It is suggested that body fat stores of infants fed Formula 305 were mobilized to permit growth of fat free tissue

KEY WORDS Skim milk infant feeding body fat depletion

From the 1940s until the mid 1960s the majority of infants in the United States were fed evaporated milk formulas or commercially prepared formulas from birth until 4 to 6 months of age and were then fed homogenized whole cow milk (1) Although data on the percentages of infants fed milk of reduced fat content are lacking it appears that in the 1970s an appreciable percentage of infants are fed skim milk or 2% milk [1] beginning at 4 to 6 months of age Reasons given by parents and physicians for use of milk of reduced fat content seem to relate primarily to the hypothesis that the overfed infant is destined to become the obese adult Thus a feeding of decreased caloric density is employed (1) as a reducing diet for an infant who is (or is believed to be) obese or (2) for the prevention of obesity Finally at least some parents and physicians appear to believe that dietary measures aimed at prevention of atherosclerosis (i e reduction in intake of cholesterol and saturated fatty acids) should be instituted as early in life as possible and therefore prefer skim to whole milk during infancy

Several theoretic considerations suggested that skim cow milk might not be as satisfactory as whole cow milk for infant feeding These will be discussed subsequently (see Discussion) Yet the absence of data on food consumption and growth of infants fed skim milk made it impossible to do more than speculate about the results of such feeding We therefore attempted to design a set of circumstances that would permit accumulation of the desired information without risk to the infant subjects Subjects known to be healthy and to be grow

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Supported by USPHS grants 1 R01 HD00383 and 1 P01 HD07578

[1] % milk is generally considered to be milk with 2% fat content and 2% added fat free milk solids

Table 1 *Composition of Formulas*

	Formula 305	Similac with Iron
Energy (kcal/100 ml)	36	67
Major constituents (g/100 ml)		
Protein	3 56	1 7[illegible]
Fat	0 [illegible]9	3 61
Carbohydrate	4 89	7 23
Mineral content per liter		
Calcium (mg)	1 300	580
Phosphorus (mg)	950	430
Sodium (mEq)	23	17
Potassium (mEq)	37	8
Chloride (mEq)	30	14
Magnesium (mg)	1[illegible]1	41
Iron (mg)	tr	1[illegible]
Copper (mg)	0 3	0 3
Vitamin content per liter		
Vitamin A (I U)	1 700	500
Thiamin (mg)	0 7	0 7
Riboflavin (mg)	1 0	1 0
Niacin (mg)	11 4	7 0
Pyridoxine (mg)	0 4	0 4
Folic acid (mg)	0 05	0 05
Pantothenate (mg)	[illegible] 0	3 0
Ascorbic acid (mg)	55	55
Vitamin D (I U)	400	400
Vitamin E (I U)	5	15

length and weight were corrected by parabolic inter polation or extrapolation utilizing three consecutive values to yield data applicable to exact ages Values applicable to 11[illegible] days were corrected using values from measurements at 84 and 140 days those applicable to 140 days were corrected using values from 11[illegible] and 168 days and those applicable to 168 days were corrected using values from 11[illegible] and 140 days

In the second study triceps and subscapular skinfold thicknesses were also measured Measurements were made as described by Fomon ([illegible]6) with the following modifications the infant was held on the mother s lap with right side adjacent to but not touching the mother s trunk the infant s left elbow was flexed 90° and the fore arm held gently against the infant s abdomen readings were made to the nearest 0 1 mm Two trained examiners measured skinfold thickness at each site three times the average of the six measurements was utilized The mean difference between examiners in measurement of triceps skinfold thickness was 0 56 mm and the standard deviation of the difference was 0 60 mm The corresponding mean difference for subscapular skinfold measurements was 0 46 mm and the standard deviation of the difference was 0 50 mm Skinfold measurements were corrected by simple linear interpolation or extrapolation using the two values closest to the desired age

RESULTS

Data concerning all infants enrolled in the study are presented in the Appendices Pre liminary analyses of the results indicated that until eliminated from the study performance of the 6 infants who failed to complete the planned period of 56 days of observation in a satisfactory manner was similar to that of the remainder of the subjects The presentation that follows concerns the 88 infants for whom complete data are available A summary of the data is presented in Table 2 Data on length and weight of individual infants are presented in Appendix I data on skinfold thicknesses are presented in Appendix II and detailed data on food intake are presented in Appendix III

Food consumption

In both studies infants fed Formula 305 con sumed greater quantities of formula than did infants fed Similac For the age interval 112 through 167 days the difference was signifi cant for males in the first study ($p<0\ 01$) and for males and females in the second study ($p<0\ 001$) Despite the greater volumes con sumed energy intake from formula by infants fed Formula 305 was less than that by infants fed Similac In each study the difference was significant ($p<0\ 001$)

Infants fed Formula 305 consumed some what greater quantities of beikost than did in fants fed Similac but the difference was not significant in either study The greater con sumption of beikost by infants fed Formula 305 was not sufficient to offset the lower in take of energy from formula Thus for infants of the same sex total energy intake in each study was significantly less for infants fed Formula 305 than for those fed Similac ($p<0\ 001$)

As in previous studies of younger infants (7) males consumed greater quantities of food than did females In the second study reported here (in which both sexes were included) total food consumption (formula and beikost) was significantly greater ($p<0\ 01$) by males than

ing normally were fed skim milk for a period of 56 days (112 through 167 days of age)

Two separate studies were carried out In both studies, performance of infants fed a slightly modified skim milk was compared with that of infants fed a commercially prepared formula (Similac with Iron[1]) Measurements included food intake and gain in length and weight The second study included in addition measurements of triceps and subscapular skinfold thickness

SUBJECTS

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Appendix I provides data on birth date weight at birth and body weight and length at age 112 days—the time of enrollment in the study described here With few exceptions available infants of the same sex were enrolled alternately by date of birth into the two feeding groups In every case the plan of study was reviewed in detail with one or both parents and written permission was obtained [2]

The studies were planned to permit 56 days of observation of each of 14 or 15 infants in each of the following feeding groups in the first study (infants born between June 18 1971 and August 30 1972) males fed Formula 305 (i e modified skim milk see Feedings) and Similac in the second study (infants born between December 28 1972 and September 14 1974) males and females fed Formula 305 and males and females fed Similac Additional infants were enrolled to replace dropouts so that in each feeding group at least 14 subjects would complete the 56 days of observation In only one group did it prove necessary to enroll more than 16 infants

Ninety four infants were enrolled and 88 were considered to have completed the planned 56 days of observation (112 through 167 days) in a satisfactory manner One male infant (1606) fed Similac was eliminated from the study after 140 days of age because it proved impossible for the authors to obtain daily weights of food consumed Another male infant (1805) fed Similac was withdrawn from study before 140 days of age because of diarrhea Two female infants fed Formula 305 were excluded before the appointment scheduled for 168 days of age one (1869) having had the feeding changed to whole cow milk and the other (1826) having been fed a soy isolate formula In neither case was the reason for the change in diet known Two infants (1782 and 1823) failed to be measured between 164 and 172 days of age (see Procedures and Methods) and were therefore considered incomplete Among the 88 infants considered to have completed the study in a satisfactory manner three (1802 2106 2122 see Appendix I) failed to be measured between 136 and 144 days of age

FEEDINGS

Table I presents the composition of the formulas fed during the first and second study It should be noted that Formula 305 is identical to skim milk except for the addition of fat soluble vitamins and a sufficient quantity of safflower oil (0 23 g/100 ml) to assure an adequate intake of linoleic acid Infants fed Formula 305 received a daily supplement providing 15 mg of iron in the form of ferrous sulfate [3] Each formula was supplied in quart cans of known gross weight A supply of formula was delivered to the family and empty partially empty or full cans were collected from the family at the time of the next delivery The returned cans were weighed to determine the quantity of formula consumed From the composition of the formula (Table I) and the quantity of formula consumed the intake of calories was calculated

Commercially prepared strained foods produced by one manufacturer[4] were permitted without restriction of variety or amount Because the infants received medical care from private practitioners in Iowa City or nearby communities or from physicians (mainly house officers) in the Child Health Clinic of the Department of Pediatrics University of Iowa a wide variety of attitudes relating to feeding of beikost (i e foods other than milk or formula) was represented Jars of beikost were purchased by the parents and the empty or partially empty jars were collected by the milk delivery team Utilizing the manufacturer s published analyses (10) the intake of calories and specific nutrients contributed by beikost could be calculated

PROCEDURES AND METHODS

With the exceptions noted previously (see Subjects) weight and length of each infant was measured as described previously (2*b*) within 4 days of each of the following ages 112 140 and 168 days Measurements of

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[2] The proposal for this research including the procedures employed in obtaining consent was reviewed and approved by the University of Iowa Committee on Research Involving Human Beings-Medicine

[3] Fer In Sol Mead Johnson Company Evansville Indiana

[4] Gerber Products Company Fremont Michigan

Table 1 *Composition of Formulas*

	Formula 305	Similac with Iron
Energy (kcal/100 ml)	36	67
Major constituents (g/100 ml)		
Protein	3 56	1 72
Fat	0 79	3 61
Carbohydrate	4 89	7 73
Mineral content per liter		
Calcium (mg)	1 300	580
Phosphorus (mg)	950	430
Sodium (mEq)	23	17
Potassium (mEq)	37	78
Chloride (mEq)	30	14
Magnesium (mg)	121	41
Iron (mg)	tr	12
Copper (mg)	0 3	0 3
Vitamin content per liter		
Vitamin A (I U)	1 700	2 500
Thiamin (mg)	0 7	0 7
Riboflavin (mg)	1 0	1 0
Niacin (mg)	11 4	7 0
Pyridoxine (mg)	0 4	0 4
Folic acid (mg)	0 05	0 05
Pantothenate (mg)	2 0	3 0
Ascorbic acid (mg)	55	55
Vitamin D (I U)	400	400
Vitamin E (I U)	5	15

length and weight were corrected by parabolic interpolation or extrapolation utilizing three consecutive values to yield data applicable to exact ages Values applicable to 112 days were corrected using values from measurements at 84 and 140 days those applicable to 140 days were corrected using values from 112 and 168 days and those applicable to 168 days were corrected using values from 112 and 140 days

In the second study triceps and subscapular skinfold thicknesses were also measured Measurements were made as described by Fomon (*b*) with the following modifications the infant was held on the mother s lap with right side adjacent to but not touching the mother s trunk the infant s left elbow was flexed 90 and the fore arm held gently against the infant s abdomen readings were made to the nearest 0 1 mm Two trained examiners measured skinfold thickness at each site three times the average of the six measurements was utilized The mean difference between examiners in measurement of triceps skinfold thickness was 0 56 mm and the standard deviation of the difference was 0 60 mm The corresponding mean difference for subscapular skinfold measurements was 0 46 mm and the standard deviation of the difference was 0 50 mm Skinfold measurements were corrected by simple linear interpolation or extrapolation using the two values closest to the desired age

RESULTS

Data concerning all infants enrolled in the study are presented in the Appendices Preliminary analyses of the results indicated that until eliminated from the study performance of the 6 infants who failed to complete the planned period of 56 days of observation in a satisfactory manner was similar to that of the remainder of the subjects The presentation that follows concerns the 88 infants for whom complete data are available A summary of the data is presented in Table 2 Data on length and weight of individual infants are presented in Appendix I data on skinfold thicknesses are presented in Appendix II and detailed data on food intake are presented in Appendix III

Food consumption

In both studies infants fed Formula 305 consumed greater quantities of formula than did infants fed Similac For the age interval 112 through 167 days the difference was significant for males in the first study ($p<0\ 01$) and for males and females in the second study ($p<0\ 001$) Despite the greater volumes consumed energy intake from formula by infants fed Formula 305 was less than that by infants fed Similac In each study the difference was significant ($p<0\ 001$)

Infants fed Formula 305 consumed somewhat greater quantities of beikost than did infants fed Similac but the difference was not significant in either study The greater consumption of beikost by infants fed Formula 305 was not sufficient to offset the lower intake of energy from formula Thus for infants of the same sex total energy intake in each study was significantly less for infants fed Formula 305 than for those fed Similac ($p<0\ 001$)

As in previous studies of younger infants (7) males consumed greater quantities of food than did females In the second study reported here (in which both sexes were included) total food consumption (formula and beikost) was significantly greater ($p<0\ 01$) by males than

ing normally were fed skim milk for a period of 56 days (112 through 167 days of age)

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by females fed Similac During the age interval 112 through 167 days total energy intake by infants fed Similac was 681 kcal/day (96 8 kcal/kg/day) for males and 609 kcal/day (94 2 kcal/kg/day) for females When energy intake was expressed as kcal/day but not when it was expressed as kcal/kg/day the difference between males and females was significant ($p<0\ 01$) Lesser sex related differences in food intake existed in the second study among infants fed Formula 305 these differences did not reach statistical significance

Of some interest is the lesser intake of beikost in the second than in the first study Average intakes by male infants fed Formula 305 and Similac in the first study averaged 304 and 248 g/day respectively Corresponding intakes in the second study were 214 and 197 g/day The explanation of this difference is uncertain but it may be attributable at least in part to general changes in attitudes in the community toward early introduction and extensive feeding of beikost

Gain in weight and length

Gain in weight was significantly less ($p<0\ 01$) by male and female infants fed Formula 305 than by those fed Similac (Table 2) During the interval 112 through 167 days of age gain in weight by infants fed Formula 305 averaged for males 13 4 g/day in the first study and 11 4 g/day in the second study gain in weight by females (second study) averaged 11 3 g/day Corresponding values for infants fed Similac were 18 1 19 3 and 16 1 g/day

Gain in weight per unit of energy intake (Table 2) was less by infants fed Formula 305 than by those fed Similac but the difference was significant ($p<0\ 02$) only in male infants in the second study A significant feeding related difference in gain in length was not demonstrated

Skinfold thickness

As previously mentioned skinfold thickness was measured only in the second study At the time of enrollment in the study (age 112 days)

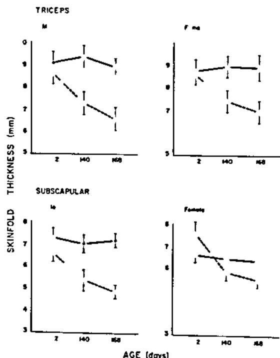


Fig 1 Triceps and subscapular skinfold thicknesses at various ages in relation to feeding by infants fed Similac (solid line) or Formula 305 (interrupted line) Formula 305 was a slightly modified skim milk with low caloric density At each age the mean and plus or minus one standard error of the mean are indicated

mean triceps skinfold thickness of males who were to be fed Formula 305 was 8 61 mm and that of males to be fed Similac was 9 08 mm This difference is not statistically significant ($p>0\ 05$) Corresponding values for mean subscapular skinfold thickness of males were 6 61 and 7 29 mm The difference is not statistically significant Similarly for females there was no statistically significant difference in mean triceps or subscapular skinfold thickness between the two groups at the time of enrollment Data on skinfold thickness of individual infants at various ages are presented in Appendix II

As may be seen from Fig 1 little change in skinfold thickness of infants fed Similac occurred between 112 and 168 days of age By contrast among male infants fed Formula 305 mean triceps skinfold thickness decreased 2 05 mm (an amount equal to 24% of the initial value) and mean subscapular skinfold thick

Table 2 *Summary of data on food consumption and growth*

Values in parentheses indicate numbers of subjects

	112–139 days		140–167 days		112–167 days		112–139 days		140–167 days		112–167 days	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
Males (first study)												
Food consumption	*Formula 305 (15)*						*Similac (15)*					
Milk or formula												
g/day	999	271	1 002	180	1 001	206	823	138	825	204	824	143
kcal/day	360	98	361	65	361	74	540	90	541	134	540	94
Strained foods												
g/day	263	114	346	144	304	115	218	165	277	176	248	165
kcal/day	186	75	244	97	215	77	159	122	198	126	178	170
Total												
g/day	1 262	265	1 348	135	1 305	158	1 041	140	1 102	205	1 072	137
kcal/day	546	105	605	68	575	60	698	100	739	139	718	96
kcal/kg/day	79.3	11.6	84.1	10.5	81.7	5.9	94.3	14.0	93.1	16.9	93.7	11.8
Gain in weight												
g/day	13.5	6.1	13.3	6.8	13.4	4.5	18.6	5.3	17.7	7.7	18.1	4.3
g/100 kcal	2.45	0.91	2.15	0.99	2.30	0.63	2.68	0.74	2.37	1.09	2.52	0.56
Gain in length												
mm/day	0.77	0.23	0.68	0.17	0.72	0.09	0.74	0.29	0.59	0.22	0.66	0.19
Males (second study)												
Food consumption	*Formula 305 (14)*						*Similac (14)*					
Milk or formula												
g/day	1 133	163	1 145	250	1 139	190	821	191	827	163	824	135
kcal/day	408	59	413	90	410	69	538	125	542	107	540	88
Strained foods												
g/day	161	110	267	138	214	118	179	79	215	97	197	86
kcal/day	117	80	192	103	155	86	129	57	152	70	141	67
Total												
g/day	1 294	164	1 413	218	1 353	175	1 000	157	1 042	146	1 021	85
kcal/day	525	84	605	102	565	86	667	102	695	98	681	56
kcal/kg/day	75.2	9.8	83.1	12.8	79.1	10.3	98.5	15.5	95.2	13.7	96.8	8.1
Gain in weight												
g/day	9.7	8.7	13.0	8.4	11.4	6.5	18.5	11.2	20.1	7.1	19.3	5.3
g/100 kcal	1.69	1.55	2.12	1.33	1.90	1.07	2.80	1.68	2.97	1.22	2.89	0.78
Gain in length												
mm/day	0.75	0.24	0.61	0.19	0.68	0.17	0.83	0.35	0.69	0.24	0.76	0.19
Females (second study)												
Food consumption	*Formula 305 (14)*						*Similac (16)*					
Milk or formula												
g/day	1 002	137	1 112	190	1 057	150	746	130	743	178	745	144
kcal/day	361	49	401	68	381	54	489	86	487	117	488	94
Strained foods												
g/day	148	68	239	105	193	82	148	85	177	102	162	90
kcal/day	109	50	166	69	138	57	110	63	131	78	121	67
Total												
g/day	1 149	107	1 351	148	1 250	117	894	94	920	127	907	97
kcal/day	470	40	567	66	519	48	599	63	618	85	609	64
kcal/kg/day	73.5	6.7	84.9	13.4	79.2	9.8	96.1	11.3	92.3	10.2	94.2	9.0
Gain in weight												
g/day	9.8	4.7	12.8	4.5	11.3	3.5	17.3	5.4	14.8	5.6	16.1	4.2
g/100 kcal	2.08	1.04	2.29	0.89	2.19	0.73	2.92	0.92	2.39	0.90	2.66	0.65
Gain in length												
mm/day	0.76	0.25	0.62	0.15	0.69	0.1						

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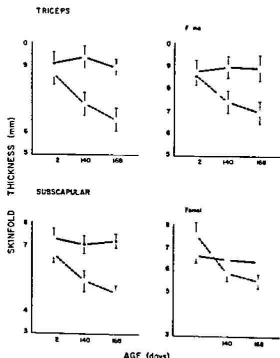


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ness decreased 1 78 mm (27%) Corresponding decreases for female infants were 1 63 mm (19%) and 2 05 mm (27%) The differences between the initial and final skinfold thicknesses for infants fed Formula 305 were significant ($p<0$ 01) for each sex

DISCUSSION

We have previously studied the influence of caloric concentration of the formula on energy intake and growth of normal fullterm infants fed ad libitum from 8 to 112 days of age (3) Between 8 and 42 days of age caloric intake and gain in weight were less by infants fed a calorically dilute formula (50 kcal/100 ml) than by those previously observed (7) who had been fed formulas of conventional caloric concentration (67 kcal/100 ml) After 42 days of age energy intake and gain in weight were nearly identical for infants fed formulas of either concentration

In the present study caloric concentration of Formula 305 was 36 kcal/100 ml and average caloric concentration of the entire diet of these infants (Formula 305 plus beikost) averaged 44 kcal/100 ml The infants did not achieve energy intakes nor rates of gain in weight equal to those of infants fed a commercially prepared formula (average caloric density of diet of Similac plus beikost was 67 kcal/100 ml) As we shall discuss the chemical composition of weight gained by infants fed Formula 305 was probably abnormal

Data presented here provide the basis for calculations of a speculative nature that may offer further insights into the relation between calorie intake and the composition of growth during infancy Various assumptions must be made to permit these calculations We believe that the insights provided by this speculative approach may be valuable although the exact numbers employed in the calculations will almost certainly be imprecise

The following assumptions have been made (*a*) chemical composition of normal growth of male infants in the interval 112 through 167 days of age is the same as that described for the male reference infant (2*c*) between 4 and 12 months of age (*b*) male infants fed Similac in the present study demonstrated normal growth, (*c*) the energy cost of deposition of fat and protein from 4 to 12 months of age was identical to that presumed to apply to such deposition between birth and 4 months of age

The estimated energy cost of tissue synthesis is based on studies of growing animals (8) which suggest that deposition of 1 g of protein requires 7 5 kcal and that deposition of 1 g of fat requires 11 6 kcal We presume that other energy requirements for growth are relatively small compared with those for synthesis of protein and fat and that such additional energy requirements were inadvertently by the nature of the experiment included in the estimates of energy required for synthesis of protein and fat Although we consider these values the best available they may not be applicable to the human subjects considered here Nevertheless if one selects other values within the most probable range (e g the limiting values of 4 kcal/g of protein and 9 kcal/g of fat or the values apparently applicable for older animals (9) 15 96 kcal/g protein and 12 96 kcal/g fat) conclusions based on the calculations to be presented here will be altered relatively little

Because each 100 g of gain in weight between 4 and 12 months of age is assumed to include 21 0 g of protein and 19 1 g of fat (2*c*) the assumed energy cost of gain of 100 g of body tissue is 379 kcal or 3 79 kcal/g

As noted in Table 2 the average gain in weight by male infants fed Similac from 112 through 167 days of age in the first study was 18 1 g/day The energy cost of growth may therefore be estimated to be 69 kcal/day (18 1 g/day×3 79 kcal/g) Because total energy intake averaged 718 kcal/day it appears that 649 kcal/day were utilized for purposes other than growth (non growth) Assuming that the infants fed Formula 305 in the first study also required 649 kcal/day for non growth one might anticipate that with total energy intake

of only 575 kcal/day no growth would occur Yet gain in weight averaged 13 4 g/day

One likely explanation for this gain in spite of relatively low energy intake is that the infants fed Formula 305 were mobilizing energy from body stores of fat to permit growth of fat free tissue Assuming that protein accounts for 26 0% of fat free body mass synthesized between 4 and 12 months of age (2c) and that energy costs of synthesis of fat free body mass may be estimated from energy costs of synthesis of protein (i e other energy costs of synthesis of fat free body mass are included in the estimated cost of synthesis of protein) energy cost of synthesis of 100 g of fat free body mass may be estimated to be 195 kcal[1] (1 95 kcal/g) Thus mobilization of 1 g of fat providing 9 kcal would permit synthesis of 4 6 g of fat free tissue with a net gain of 3 6 g in body weight

If the hypothesis of mobilization of fat to permit synthesis of fat free tissue were correct we believed that skinfold measurements would reflect a sharp decrease in skinfold thickness of infants fed Formula 305 It was for this reason that the second study was carried out and in fact as may be seen from Fig 1 a dramatic decrease in skinfold thickness was recorded

As already mentioned the mean decrease in skinfold thickness of male infants fed Formula 305 between 112 and 168 days of age averaged 2 05 mm for triceps and 1 78 mm for the subscapular site These changes amounted to 24% and 27% respectively of the mean skinfold thickness recorded at 112 days of age Because it is likely that there was a greater decrease in the fat than in the non fat components of the skinfolds of infants fed Formula 305 a decrease of 24 to 27% in skinfold thickness suggests an even greater decrease in subcutaneous fat content at these sites

We have attempted to estimate the percentage of fat lost from the body by male infants fed Formula 305 in the second study through calculations unrelated to skinfold thickness For this approach it has been assumed that energy expenditures for nongrowth were similar for the two feeding groups The mobilization of 9 g of fat daily by the infants fed Formula 305 would provide 81 kcal/day Thus available energy would be 646 kcal/day (i e energy intake of 565 kcal/day for males in the second study plus 81 kcal/day from fat stores) Synthesis of fat free tissue of 20 4 g/day would be required the observed gain in weight of 11 4 g/day plus 9 0 g/day to replace the loss in weight from mobilization of fat Synthesis of fat free tissue would require 40 kcal (20 4 g ×1 95 kcal/g=40 kcal) and therefore 606 kcal/day (646 kcal−40 kcal=606 kcal) would be available for non growth—a value similar to that calculated for non growth (608 kcal/day[2]) for males fed Similac in the second study

The loss of 504 g of fat (9 g/day for 56 days) amounts to approximately 28% of the estimated 1 820 g present in the male reference infant at age 4 months (2c) Therefore one may speculate that approximately 28% of body fat content may have been lost in the 56 days of study

It is of course possible that energy expenditures for non growth may have been less for infants fed Formula 305 than for those fed Similac The study reported here does not provide clues about relative expenditures of energy for non growth in the two feeding groups

Although there can be little question that infants fed Formula 305 lost body fat the calculations are probably not quantitatively correct Even if they were however it would be difficult to estimate how long it would take for body stores of fat to become depleted The rate of depletion would be likely to decrease with increasing age because a larger percentage of calories would be derived from beikost as the infant got older Nevertheless in some

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[1] 100 g fat free tissue includes 6 0 g protein .6 0 g× 7 5 kcal/g=195 kcal

[2] 681 kcal−19 3 g×3 79 kcal/g=608 kcal

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DISCUSSION

We have previously studied the influence of caloric concentration of the formula on energy intake and growth of normal fullterm infants fed ad libitum from 8 to 112 days of age (3) Between 8 and 42 days of age caloric intake and gain in weight were less by infants fed a calorically dilute formula (50 kcal/100 ml) than by those previously observed (7) who had been fed formulas of conventional caloric con centration (67 kcal/100 ml) After 42 days of age energy intake and gain in weight were nearly identical for infants fed formulas of either concentration

In the present study, caloric concentration of Formula 305 was 36 kcal/100 ml and average caloric concentration of the entire diet of these infants (Formula 305 plus beikost) averaged 44 kcal/100 ml The infants did not achieve energy intakes nor rates of gain in weight equal to those of infants fed a commercially pre pared formula (average caloric density of diet of Similac plus beikost was 67 kcal/100 ml) As we shall discuss the chemical composition of weight gained by infants fed Formula 305 was probably abnormal

Data presented here provide the basis for calculations of a speculative nature that may offer further insights into the relation between calorie intake and the composition of growth during infancy Various assumptions must be made to permit these calculations We believe that the insights provided by this speculative approach may be valuable although the exact numbers employed in the calculations will almost certainly be imprecise

The following assumptions have been made (*a*) chemical composition of normal growth of male infants in the interval 112 through 167 days of age is the same as that described for the ' male reference infant (2*c*) between 4 and 12 months of age (*b*) male infants fed Similac in the present study demonstrated normal growth (*c*) the energy cost of deposition of fat and protein from 4 to 12 months of age was identical to that presumed to apply to such de position between birth and 4 months of age

The estimated energy cost of tissue synthe sis is based on studies of growing animals (8) which suggest that deposition of 1 g of protein requires 7 5 kcal and that deposition of 1 g of fat requires 11 6 kcal We presume that other energy requirements for growth are relatively small compared with those for synthesis of protein and fat and that such additional energy requirements were inadvertently by the na ture of the experiment included in the esti mates of energy required for synthesis of pro- tein and fat Although we consider these values the best available they may not be applicable to the human subjects considered here Nevertheless if one selects other values within the most probable range (e g the limit ing values of 4 kcal/g of protein and 9 kcal/g of fat or the values apparently applicable for older animals (9) 15 96 kcal/g protein and 12 96 kcal/g fat) conclusions based on the cal culations to be presented here will be altered relatively little

Because each 100 g of gain in weight be tween 4 and 12 months of age is assumed to include 21 0 g of protein and 19 1 g of fat (2*c*) the assumed energy cost of gain of 100 g of body tissue is 379 kcal or 3 79 kcal/g

As noted in Table 2 the average gain in weight by male infants fed Similac from 112 through 167 days of age in the first study was 18 1 g/day The energy cost of growth may therefore be estimated to be 69 kcal/day (18 1 g/day×3 79 kcal/g) Because total energy in take averaged 718 kcal/day it appears that 649 kcal/day were utilized for purposes other than growth (non growth) Assuming that the infants fed Formula 305 in the first study also required 649 kcal/day for non growth one might anticipate that with total energy intake

of only 575 kcal/day no growth would occur Yet gain in weight averaged 13 4 g/day

One likely explanation for this gain in spite of relatively low energy intake is that the infants fed Formula 305 were mobilizing energy from body stores of fat to permit growth of fat free tissue Assuming that protein accounts for 26 0% of fat free body mass synthesized between 4 and 12 months of age (2*c*) and that energy costs of synthesis of fat free body mass may be estimated from energy costs of synthesis of protein (i e other energy costs of synthesis of fat free body mass are included in the estimated cost of synthesis of protein) energy cost of synthesis of 100 g of fat free body mass may be estimated to be 195 kcal[1] (1 95 kcal/g) Thus mobilization of 1 g of fat providing 9 kcal would permit synthesis of 4 6 g of fat free tissue with a net gain of 3 6 g in body weight

If the hypothesis of mobilization of fat to permit synthesis of fat free tissue were correct we believed that skinfold measurements would reflect a sharp decrease in skinfold thickness of infants fed Formula 305 It was for this reason that the second study was carried out and in fact as may be seen from Fig 1 a dramatic decrease in skinfold thickness was recorded

As already mentioned the mean decrease in skinfold thickness of male infants fed Formula 305 between 112 and 168 days of age averaged 2 05 mm for triceps and 1 78 mm for the subscapular site These changes amounted to 24% and 27% respectively of the mean skinfold thickness recorded at 112 days of age Because it is likely that there was a greater decrease in the fat than in the non fat components of the skinfolds of infants fed Formula 305 a decrease of 24 to 27% in skinfold thickness suggests an even greater decrease in subcutaneous fat content at these sites

We have attempted to estimate the percentage of fat lost from the body by male infants fed Formula 305 in the second study through calculations unrelated to skinfold thickness For this approach it has been assumed that energy expenditures for nongrowth were similar for the two feeding groups The mobilization of 9 g of fat daily by the infants fed Formula 305 would provide 81 kcal/day Thus available energy would be 646 kcal/day (i e energy intake of 565 kcal/day for males in the second study plus 81 kcal/day from fat stores) Synthesis of fat free tissue of 20 4 g/day would be required the observed gain in weight of 11 4 g/day plus 9 0 g/day to replace the loss in weight from mobilization of fat Synthesis of fat free tissue would require 40 kcal (20 4 g ×1 95 kcal/g=40 kcal) and therefore 606 kcal/day (646 kcal−40 kcal=606 kcal) would be available for non growth—a value similar to that calculated for non growth (608 kcal/day[2]) for males fed Similac in the second study

The loss of 504 g of fat (9 g/day for 56 days) amounts to approximately 28% of the estimated 1 820 g present in the male reference infant at age 4 months (2*c*) Therefore one may speculate that approximately 28% of body fat content may have been lost in the 56 days of study

It is of course possible that energy expenditures for non growth may have been less for infants fed Formula 305 than for those fed Similac The study reported here does not provide clues about relative expenditures of energy for non growth in the two feeding groups

Although there can be little question that infants fed Formula 305 lost body fat the calculations are probably not quantitatively correct Even if they were however it would be difficult to estimate how long it would take for body stores of fat to become depleted The rate of depletion would be likely to decrease with increasing age because a larger percentage of calories would be derived from beikost as the infant got older Nevertheless in some

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[1] 100 g fat free tissue includes 26 0 g protein 26 0 g× 7 5 kcal/g=195 kcal

[2] 681 kcal−19 3 g×3 79 kcal/g=608 kcal

infants eventual depletion of fat stores might occur, thereby placing the infant in considerable jeopardy with respect to his ability to withstand a prolonged serious illness An additional cause for concern is the possibility that consumption of extremely large volumes of food in the apparent attempt to achieve adequate intakes of energy might establish undesirable habits of eating If excessive gastric filling were to become recognized by the infant as the signal to discontinue eating the infant might become quite slim while skim milk remained a major dietary component Later on however with access to foods of greater caloric density eating to the point of achieving similar gastric filling might lead to obesity

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10 Professional Communications Department *Nutrient values of Gerber baby foods* Gerber Products Co Fremont Michigan 1972

Submitted April 10 1976
Accepted June 30 1976

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APPENDIX I–III

Appendix 1 *Weights and lengths of individual infants at various ages*

Value interpolated from measurement made more than 4 days from stated age (see text)

Subject	Birth date	Birth weight (g)	112 days		140 days		168 days	
			Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)
MALES								
Formula 305 1st study								
1609	8 9 71	4 040	7 897	65 7	8 703	68 4	9 210	70 1
1610	8 13 71	3 125	6 645	63 1	6 8?5	64 7	7 275	66 5
1611	8 23 71	3 890	7 164	63 2	7 431	66 2	7 7?2	67 7
161?	8 31 71	4 150	7 979	67 6	8 300	69 6	8 677	71 5
166?	10-11 71	3 800	6 358	62 8	6 816	65 7	7 008	67 2
1665	11 4 71	3 160	6 133	61 5	6 640	63 0	6 715	64 8
1666	11 12 71	3 1?0	5 600	59 9	6 030	62 2	6 075	63 3
1667	11 17 71	3 490	6 964	64 4	7 459	67 4	8 099	69 0
1672	1 28 72	3 300	6 850	63 1	7 185	6? 5	7 4?0	6? 5
1080	4 10-72	3 040	5 947	61 9	6 ?61	63 8	6 697	65 7
17?1	4-11 72	2 560	6 808	60 5	6 9?7	6? 0	7 3?5	65 0
17??	4 ?4 7?	2 900	6 149	6? 6	6 430	63 9	6 691	66 3
174?	4-27 72	2 890	6 087	59 3	6 557	6? 3	7 080	64 2
17?7	7 6-72	4 100	7 337	65 9	7 816	67 8	8 ?87	69 4
17 8	8 19 72	3 640	6 306	63 7	6 516	65 0	7 ?08	67 7
Similac 1st study								
1604	6-18 71	3 080	7 450	60 8	7 7?5	63 4	8 4?0	65 5
1605	7 6-71	3 865	7 670	64 9	8 398	66 0	9 036	66 7
1606	7 6-71	4 450	7 571	66 1	7 975	67 0	–	–
1613	9-11 71	4 040	7 460	64 6	7 857	67 2	8 504	67 8
1614	9-14 71	3 850	7 387	65 2	7 96?	67 7	8 555	69 3
1659	10-?5-71	3 710	7 976	64 6	8 698	67 0	9 341	69 1
1668	11 ?5 71	4 0?0	8 440	68 6	8 888	70 8	9 097	73 0
1660	11 30-71	3 900	6 952	63 9	7 670	64 7	8 007	65 9
1669	1? ?3 71	3 100	6 003	59 7	6 406	61 4	6 787	63 2
1671	1 12 72	3 680	6 97?	63 9	7 314	64 4	7 858	65 9
1079	3 3 7	3 540	7 210	63 ?	7 74?	65 0	8 300	67 3
1 ?3	5- 5-72	3 785	7 120	64 5	7 470	67 1	7 990	68 6
17 4	5-14-7?	3 950	6 756	64 5	7 270	66 1	7 879	69 0
17 5	5 6-7?	3 300	6 045	6? 2	6 605	64 4	7 060	65 6
1748	8-19-7?	4 170	7 041	64 5	7 695	67 5	7 594	68 7
17 9	8-30-7?	4 140	7 150	64 0	7 739	67 4	8 436	69 1
Formula 305 2nd study								
178	3-?3-73	3 ?70	7 515	64 7	7 951	66 2	8 114	68 0
1809	7 17 73	3 590	6 693	64 1	6 855	65 7	7 256	67 8
1810	7 ?0-73	3 330	5 973	61 5	6 265	63 3	6 673	64 6
1811	7 ?6-73	4 440	8 271	68 4	8 906	69 5	9 131	70 3
1813	8 9-73	3 010	6 725	65 3	6 736	67 7	7 2?0	68 2
1815	9-14-73	3 495	6 559	6? 1	6 958	64 4	7 63?	66 2
185?	? ?6-74	3 970	6 791	6? 9	7 301	65 1	7 556	67 0
1884	4- ? 74	3 800	6 931	6? 4	7 3?0	64 2	7 237	65 9
1886	4- 8 74	4 440	7 4?6	67 5	8 086	69 7	8 592	71 3
1891	6-16-74	2 780	6 ?49	6? 3	6 4?8	63 6	7 073	65 6
189?	7 14-74	3 350	6 980	60 6	7 09?	63 1	7 634	65 6
?1	8-10-74	4 110	8 03?	67 1	8 375	71 1	8 975	73 0
?1.3	8- 0-74	3 545	6 537	60 4	6 771	62 5	7 063	64 3
?1.5	8-31 74	4 180	6 907	65 0	7 030	67 0	7 197	69 3
?1 6	9-14 74	3 180	5 944	61 5	5 710	63 6	5 705	65 3

infants, eventual depletion of fat stores might occur thereby placing the infant in considerable jeopardy with respect to his ability to withstand a prolonged serious illness An additional cause for concern is the possibility that consumption of extremely large volumes of food in the apparent attempt to achieve adequate intakes of energy might establish undesirable habits of eating If excessive gastric filling were to become recognized by the infant as the signal to discontinue eating the infant might become quite slim while skim milk remained a major dietary component Later on however with access to foods of greater caloric density eating to the point of achieving similar gastric filling might lead to obesity

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Submitted April 10 1976
Accepted June 30 1976

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Appendix II *Skinfold thickness (mm) of individual infants at various ages*

Value interpolated from measurement more than 4 days from stated age (see text)

Subject	11? days		140 days		168 days	
	Triceps	Subscapular	Triceps	Subscapular	Triceps	Subscapular
MALES						
Formula 305 2nd study						
178?	11 7	10 2	10 6	7 ?	10 4	6 9
1809	11 1	6 9	9 6	4 5	8 4	5 2
1810	8 8	5 5	5 7	3 6	4 3	3 8
1811	11 1	11 2	11 6	10 5	9 7	6 9
1813	7 6	5 8	6 3	5 0	5 1	3 9
1815	11 4	7 9	8 2	6 0	9 6	6 6
185?	7 5	6 1	6 9	4 8	7 9	4 6
1884	9 ?	6 7	9 1	7 1	6 0	4 7
1886	8 2	5 0	5 8	4 5	4 6	4 6
1891	7 ?	6 3	6 0	4 7	6 6	4 6
189?	9 2	7 0	7 7	6 1	6 5	5 9
21??	8 7	6 5	7 8	4 3	7 3	4 1
?123	7 1	6 5	6 8	5 9	6 9	5 5
?1?5	6 3	6 1	4 8	4 3	4 7	3 9
?1 6	7 2	5 0	5 4	4 0	4 3	3 3
Similac 2nd study						
1783	6 8	5 0	7 8	5 9	9 5	6 8
1784	8 8	6 9	10 3	6 9	8 0	7 5
1801	8 6	7 8	10 6	7 7	9 9	9 3
180?	8 9	6 4	9 5	7 0	8 ?	7 1
1803	8 0	5 9	8 5	6 2	7 6	6 3
1804	6 6	5 ?	9 1	5 2	8 3	5 1
1805	10 4	6 ?	–	–	–	–
1806	10 9	6 9	–	–	8 6	7 2
1841	10 6	10 3	11 0	9 0	11 3	9 7
1094	7 0	6 2	7 2	6 5	8 6	6 3
1844	11 4	10 0	12 5	9 7	9 9	7 4
1845	8 3	7 2	7 9	6 0	7 1	6 7
1846	8 0	7 0	6 9	5 5	9 1	6 3
1850	12 8	9 7	11 6	8 9	11 2	7 9
1851	10 4	7 6	8 4	7 1	7 4	6 9
FEMALES						
Formula 305 2nd study						
1769	10 6	7 0	10 9	5 7	10 0	5 9
1770	8 4	8 0	6 8	5 8	6 0	5 0
1771	7 2	6 5	5 2	6 0	5 4	6 0
18 1	10 0	10 6	9 6	7 2	11 3	7 0
18	10 5	8 6	6 7	6 7	8 1	6 9
18 3	9 9	10 5	7 4	6 9	6 4	6 9
18 4	6 7	5 9	5 9	4 5	5 1	4 4
18 5	8 4	5 8	10 3	4 4	6 6	4 6
18 6	8 9	6 6	7 2	4 8	–	–
18 7	6 3	5 4	5 ?	4 3	4 7	4 1
1869	7 9	8 ?	6 9	5 4	–	–
187	9 6	1? 6	7 9	8 4	7 6	7 0
1875	7 6	6 6	6 9	5 7	6 1	5 1
1876	8 5	7 8	7 9	5 9	7 0	5 2
?10	6 6	6 1	5 4	5 1	5 4	4 3
106	9 7	6 0	7 0	4 7	5 9	4 1
107	9 6	8 5	8 0	7 3	7 7	7 1

App I Cont

Subject	Birth date	Birth weight (g)	112 days Weight (g)	112 days Length (cm)	140 days Weight (g)	140 days Length (cm)	168 days Weight (g)	168 days Length (cm)
Similac 2nd study								
1783	4 10-73	3 500	6 520	62 5	6 970	64 6	7 651	67 4
1784	4 14 73	3 375	6 060	62 0	6 735	63 4	7 172	65 7
1801	4 22 73	4 010	6 850	64 9	7 567	67 1	8 129	69 3
1802	4 28 73	2 810	6 266	59 7	7 315*	64 8*	7 938	66 7
1803	5 10-73	4 255	7 203	62 3	7 660	63 9	8 011	66 5
1804	5 12 73	2 880	5 650	62 2	5 838	64 3	6 343	65 8
1805	5 21 73	3 430	6 214	62 7	–	–	–	–
1806	5 26-73	3 646	6 467	62 7	6 894	65 7	7 280	67 4
1841	9 21 73	3 070	6 494	59 0	7 104	61 5	7 580	64 2
1094	10-30-73	3 435	6 330	62 8	6 816	64 7	7 682	65 1
1844	11 16-73	3 070	7 101	60 9	8 191	63 6	8 676	65 1
1845	12 4-73	3 280	6 878	62 7	7 375	65 0	7 716	66 2
1846	12 20-73	3 830	6 204	60 2	6 500	62 8	7 054	64 4
1850	2 2 74	3 205	6 218	59 1	6 642	61 5	7 189	63 4
1851	2 12 74	3 710	7 180	65 8	7 053	66 6	8 110	69 2
FEMALES								
Formula 305 2nd study								
1769	6- 8 73	2 925	6 640	62 3	6 953	63 7	7 256	65 5
1770	6-29 73	3 340	6 840	61 9	7 074	63 6	7 368	65 0
1771	6-29 73	3 750	6 327	63 0	6 611	65 5	6 901	67 1
1821	7 6-73	4 000	7 370	64 6	7 618	66 5	8 163	68 0
1822	7 10-73	3 590	6 884	62 4	7 308	65 2	7 846	67 1
1823	7 13 73	3 040	6 394	58 9	6 811	61 7	6 954*	62 4*
1824	7 13 73	3 120	5 704	59 3	5 604	62 4	5 930	63 5
1825	7 22 73	3 730	5 618	60 5	5 974	61 4	6 318	63 3
1826	7 27 73	3 120	5 496	59 9	5 990	62 3	–	–
1827	8 1 73	3 560	6 040	61 9	6 350	63 7	6 615	65 2
1869	3 4 74	3 375	5 905	58 3	6 365	61 0	–	–
1872	4 19-74	3 180	6 714	61 4	7 056	62 9	7 288	64 4
1875	5 13 74	5 420	5 251	58 6	5 628	60 6	5 886	62 6
1876	5 23 74	2 820	5 471	58 2	5 689	60 0	6 267	62 0
2102	5 25 74	2 820	5 670	59 1	6 089	62 3	6 581	64 7
2106	8 9 74	3 870	7 133	65 0	7 332*	67 6*	7 528	70 1
2107	9- 2 74	3 370	6 455	60 2	6 674	62 9	7 030	64 0
Similac 2nd study								
1713	12 28 72	3 770	7 069	63 6	7 747	65 9	8 369	68 4
1692	1 21 73	3 440	5 922	62 8	6 298	64 1	6 838	66 7
1693	1 26-73	3 460	6 788	63 5	7 263	65 1	7 570	66 7
1714	2 6-73	3 265	6 348	61 4	6 579	64 1	7 029	66 1
1715	2 22 73	2 895	5 971	62 6	6 756	64 8	7 105	67 0
1694	2 23 73	2 970	5 870	60 0	6 225	61 4	6 530	63 8
1761	3 14 73	3 740	6 232	60 4	6 770	64 2	7 130	65 3
1762	4-28 73	3 080	5 903	59 7	6 523	62 4	7 189	65 1
1764	4 30-73	3 210	5 730	63 2	6 191	63 9	6 562	65 5
1765	4 30-73	3 430	6 950	63 3	7 494	64 9	7 969	66 8
1766	5 10-73	3 040	6 018	61 4	6 472	62 4	6 951	64 6
1767	5 11 73	2 725	5 861	60 7	6 217	63 0	6 609	64 6
1829	9-11 73	3 050	5 349	62 3	6 009	64 3	6 543	66 8
1830	9 14-73	2 755	5 030	58 0	5 337	60 0	5 786	61 8
1831	9 18 73	2 670	6 220	60 4	6 652	63 2	6 667	64 3
1832	9 30-73	3 010	5 193	58 7	5 735	60 7	6 009	63 2

Appendix II *Skinfold thickness (mm) of individual infants at various ages*

Value interpolated from measurement more than 4 days from stated age (see text)

Subject	112 days		140 days		168 days	
	Triceps	Subscapular	Triceps	Subscapular	Triceps	Subscapular
MALES						
Formula 305 2nd study						
178?	11 7	10 2	10 6	7 2	10 4	6 9
1809	11 1	6 9	9 6	4 5	8 4	5 2
1810	8 8	5 5	5 7	3 6	4 3	3 8
1811	11 1	11 2	11 6	10 5	9 7	6 9
1813	7 6	5 8	6 3	5 0	5 1	3 9
1815	11 4	7 9	8 2	6 0	9 6	6 6
1852	7 5	6 1	6 9	4 8	7 9	4 6
1884	9 2	6 7	9 1	7 1	6 0	4 7
1886	8 ?	5 0	5 8	4 5	4 6	4 6
1891	7 ?	6 3	6 0	4 7	6 6	4 6
189?	9 2	7 0	7 7	6 1	6 5	5 9
212?	8 7	6 5	7 8	4 3*	7 3	4 1
?123	7 1	6 5	6 8	5 9	6 9	5 5
?1?5	6 3	6 1	4 8	4 3	4 7	3 9
?1 6	7 2	5 0	5 4	4 0	4 3	3 3
Similac 2nd study						
1783	6 8	5 0	7 8	5 9	9 5	6 8
1784	8 8	6 9	10 3	6 9	8 0	7 5
1801	8 6	7 8	10 6	7 7	9 9	9 3
180?	8 9	6 4	9 5	7 0	8 2	7 1
1803	8 0	5 9	8 5	6 ?	7 6	6 3
1804	6 6	5 2	9 1	5 ?	8 3	5 1
1805	10 4	6 ?	–	–	–	–
1806	10 9	6 9	–	–	8 6	7 2
1841	10 6	10 3	11 0	9 0	11 3	9 7
1094	7 0	6 2	7 ?	6 5	8 6	6 3
1844	11 4	10 0	12 5	9 7	9 9	7 4
1845	8 3	7 2	7 9	6 0	7 1	6 7
1846	8 0	7 0	6 9	5 5	9 1	6 3
1850	12 8	9 7	11 6	8 9	11 2	7 9
1851	10 4	7 6	8 4	7 1	7 4	6 9
FEMALES						
Formula 305 2nd study						
1769	10 6	7 0	10 9	5 7	10 0	5 9
1770	8 4	8 0	6 8	5 8	6 0	5 0
1771	7 ?	6 5	5 2	6 0	5 4	6 0
18?1	10 0	10 6	9 6	7 2	11 3	7 0
18?	10 5	8 6	6 7	6 7	8 1	6 9
18 3	9 9	10 5	7 4	6 9	6 4*	6 9
18 4	6 7	5 9	5 9	4 5	5 1	4 4
18 5	8 4	5 8	10 3	4 4	6 6	4 6
18?6	8 9	6 6	7 2	4 8	–	–
18?7	6 3	5 4	5 2	4 3	4 7	4 1
1869	7 9	8 ?	6 9	5 4	–	–
187?	9 6	1? 6	7 9	8 4	7 6	7 0
1875	7 6	6 6	6 9	5 7	6 1	5 1
1876	8 5	7 8	7 9	5 9	7 0	5 2
10	6 6	6 1	5 4	5 1	5 4	4 3
106	9 7	6 0	7 0	4 7	5 9	4 1
107	9 6	8 5	8 0	7 3	7 7	7 1

App II Cont

Subject	112 days		140 days		168 days	
	Triceps	Subscapular	Triceps	Subscapular	Triceps	Subscapular
Similac 2nd study						
1713	–	–	–	–	–	–
1692	–	–	–	–	–	–
1693	–	–	–	–	–	–
1714	–	–	–	–	–	–
1715	8 4	5 6	8 3	5 5	7 0	5 2
1694	9 0	7 3	9 2	7 4	8 6	7 5
1761	9 2	7 3	9 6	7 4	10 2	8 4
1762	12 1	7 3	10 7	7 4	11 3	7 1
1764	7 7	5 5	8 9	5 5	8 0	5 9
1765	9 3	6 9	10 7	6 1	11 4	5 9
1766	9 0	6 7	10 2	6 3	6 5	6 4
1767	10 3	7 4	10 9	7 7	11 9	6 6
1829	7 0	4 9	7 1	5 3	7 4	4 9
1830	8 8	7 0	7 1	6 6	7 8	6 1
1831	9 4	8 2	9 0	7 0	9 7	7 2
1832	5 1	5 0	5 6	5 2	6 8	5 1

Appendix III *Average daily intake of formula and beikost by individual infants*

Subject	Formula intake (g)		Beikost intake (g)							
			11?–139 days				140–167 days			
	112–139 days	140–167 days	Total	Protein	Fat	CHO	Total	Protein	Fat	CHO
MALES										
Formula 305 1st study										
1609	891	833	462	6 3	4 1	66 9	571	7 ?	5 5	8? 4
1610	617	950	213	3 0	1 6	26 8	536	7 4	4 9	69 7
1611	1 198	1 07?	378	3 7	1 6	59 0	?99	3 5	? 2	44 5
161?	1 017	838	505	7 8	4 9	61 4	443	4 9	2 2	65 6
166	1 346	1 035	75	0 9	0 8	1? 6	79	0 7	0 6	13 1
1665	1 133	1 431	186	2 4	1 5	25 6	116	1 ?	1 1	17 4
1666	901	881	?38	3 1	1 9	36 1	?95	5 ?	3 2	44 3
1667	1 604	1 336	?03	? ?	1 4	28 9	?45	3 ?	? 0	3? 2
167?	886	843	?53	2 3	1 3	40 4	423	4 3	3 4	59 1
1080	1 033	966	157	1 8	0 9	?5 6	350	3 3	? 1	59 0
1721	984	1 070	248	2 4	0 9	39 5	390	4 9	2 6	54 0
17??	877	854	?16	1 5	0 8	37 3	329	2 4	1 4	57 ?
174	1 057	988	198	3 0	3 0	27 1	353	7 4	4 7	43 5
17?7	983	1 080	295	2 7	1 3	48 7	234	2 2	1 1	39 ?
17?8	465	857	314	3 7	? 8	47 ?	525	6 6	4 9	73 9
Similac 1st study										
1604	904	973	42	1 5	? 4	3 ?	129	2 9	3 4	17 4
1605	931	771	?16	2 8	? 3	3? ?	179	2 9	2 ?	?4 5
1606	833	–	184	? 7	? 1	?0 7	–	–	–	–
1613	681	9?4	165	1 8	1 0	25 8	344	4 0	2 7	51 3
1614	500	551	586	4 6	? 7	96 0	653	17 4	6 9	84 1
1659	918	795	164	? 6	1 0	?0 4	?95	4 8	3 3	38 2
1660	866	6 ?	15?	1 0	0 6	25 6	384	? 1	1 ?	61 5
1668	730	887	340	4	1 3	55 9	94	4 1	2 1	44 5
1669	604	730	234	1 9	1 0	38 8	?11	2 1	1 4	32 0
1671	938	1 3?9	89	1	0 5	1? 4	152	? 1	0 9	?0 9
1079	899	996	?09	4 5	? 9	?7 0	273	4 4	2 5	35 6
17 3	990	831	89	0 8	0 4	14 6	173	1 9	1 3	25 9
17?4	845	1 0?3	155	1 9	1 ?	21 2	?13	4 8	? 5	?9 ?
17-5	91?	675	164	1 9	1 ?	25 5	43	0 6	0 7	5 9
1748	800	6?1	89	0 6	0 3	15 6	164	? ?	1 1	?3 7
17 9	8?8	646	583	5 6	3 ?	95 2	651	1? 1	5 4	93 7
Formula 305 2nd study										
1 8	767	1 643	161	- 0	1 1	-5 9	335	3 6	? 5	5? 7
1809	1 141	1 177	1 5	1 4	1 3	19 3	69	2 0	1 7	44 9
1810	955	1 11?	?70	?	1 0	4 6	351	? 6	1 3	55 4
1811	1 39	1 7?1	55	0 4	0 5	10 1	83	0 5	0 6	16 4
1813	1 ?49	1 084	58	2 1	1 3	5 ?	314	11 9	6 9	3? 0
1815	95?	9 9	77	3 0	1 3	44 8	483	11 8	7 4	70 1
185	1 039	1 419	35	3 0	? 6	31 1	?67	4 0	3 3	36 4
1884	1 300	1 350	1?8	1 5	1 1	0 3	04	2 5	1 6	32 4
1886	1 64	1 084	4?3	5 5	3 7	64	476	9 7	5 4	61 4
1891	954	813	16	3	1 4	30 ?	478	7 3	3 3	6? 5
189	1 15	1 19	53	0 9	0 5	7 ?	174	2 8	1 1	?0 1
1	1 371	1 366	81	0 9	0 8	11	13?	1 1	0 7	17 6
1 3	1 068	988	153	1 7	0 7	9 3	?40	1 0	1 0	43 5
1 5	1 1 5	877	57	0 3	0 1	10 1	101	0 6	0 5	17 8
1 6	899	9 5	118	1 5	0 9	15 8	173	- 7	1	18 1

App III Cont

	Formula intake (g)		Beikost intake (g)							
			112–139 days				140–167 days			
Subject	112–139 days	140–167 days	Total	Protein	Fat	CHO	Total	Protein	Fat	CHO
Similac 2nd study										
1783	807	843	167	1 4	0 6	25 9	208	2 1	1 6	28 3
1784	709	786	231	2 0	1 0	38 2	296	2 3	1 2	48 4
1801	534	1 048	270	2 8	1 3	43 1	275	4 9	2 3	39 6
1802	1 132	1 213	30	0 2	0 1	5 8	28	0 3	0 2	4 5
1803	857	709	209	1 3	0 6	38 1	229	1 4	0 7	40 8
1804	514	641	212	2 6	1 7	29 8	317	3 3	2 1	45 7
1805	–	–	–	–	–	–	–	–	–	–
1806	709	747	273	2 2	1 2	45 0	352	3 2	1 7	56 6
1841	758	644	178	1 4	0 7	29 4	255	2 7	1 0	41 7
1094	1 111	718	122	2 6	1 3	16 7	97	2 9	1 4	10 6
1844	950	880	33	0 2	0 0	4 8	56	0 8	0 3	7 8
1845	806	921	162	1 6	0 9	24 8	255	6 1	3 7	32 3
1846	716	935	132	1 2	0 7	21 4	179	1 7	0 7	29 3
1850	1 060	684	219	2 8	1 4	31 5	192	2 7	2 1	24 6
1851	825	812	271	2 5	1 3	42 1	268	2 2	1 7	38 5
FEMALES										
Formula 305 2nd study										
1769	914	987	190	2 0	1 5	30 2	259	3 0	2 0	41 ?
1770	1 019	1 110	120	1 1	0 6	21 2	150	1 7	1 3	22 6
1771	1 115	1 171	177	2 2	1 2	27 9	278	3 9	2 2	40 1
1821	1 216	1 360	65	0 7	0 7	10 1	66	1 2	0 6	6 6
1822	1 0?8	1 162	220	1 9	1 0	35 9	319	3 0	1 5	53 ?
1823[a]	900	1 001	14	0 2	0 1	2 2	4	0 0	0 0	0 6
1824	972	1 079	95	0 7	0 8	17 7	169	0 8	1 0	32 5
1825	964	1 352	186	3 8	2 3	27 0	273	4 3	3 1	37 5
1826	985	–	277	2 5	1 7	42 8	–	–	–	–
1827	856	1 020	286	2 3	1 3	45 0	327	3 8	2 6	51 4
1869	981	–	142	1 4	0 8	24 2	–	–	–	–
1872	965	689	170	2 0	1 2	27 6	437	10 9	1 3	43 4
1875	784	889	207	2 9	1 7	31 6	374	4 2	2 1	57 0
1876	872	1 016	133	1 2	0 7	20 6	207	5 0	2 8	?6 8
2102	9?2	1 133	89	0 8	0 4	16 3	91	0 8	0 5	16 2
2106	1 216	1 232	51	0 7	0 4	7 3	180	3 9	1 6	19 5
2107	1 185	1 369	80	1 0	0 2	8 7	212	2 2	1 3	30 9
Similac 2nd study										
1713	760	1 027	167	0 7	0 4	32 2	13?	0 5	0 5	26 5
1692	780	574	242	1 9	1 1	44 1	254	2 6	1 6	38 1
1693	802	849	103	1 6	1 3	14 2	107	2 3	1 5	14 0
1714	1 021	1 026	–	–	–	–	–	–	–	–
1715	912	902	21	0 2	0 1	2 9	170	1 4	0 5	16 9
1694	707	481	228	3 8	2 5	31 7	244	2 8	2 0	36 4
1761	952	852	135	1 2	1 2	19 8	321	5 5	5 ?	45 3
1762	772	921	22	0 2	0 1	3 8	56	0 5	0 2	9 7
1764	597	650	161	1 5	0 8	27 6	217	7 6	3 7	?? 1
1765	744	815	174	1 7	1 0	29 5	168	0 9	0 5	27 0
1766	651	663	136	7 4	2 2	15 9	134	1 1	0 6	23 6
1767	520	441	288	3 9	2 0	41 9	358	8 7	4 5	50 5
1829	642	579	253	8 8	? 8	31 3	?96	5 4	? 2	44 6
1830	699	711	154	1 8	0 7	24 7	158	1 7	0 6	25 2
1831	721	762	83	0 7	0 4	14 2	54	0 4	0 2	9 0
1832	661	662	193	2 2	0 7	28 3	219	? 3	0 8	32 4

[a] Subject who did not complete study for various reasons (see text)

Acta Pædiatr Scand 66 31–37 1977

CLINICAL AND IMMUNOLOGICAL ASPECTS OF FOOD ALLERGY IN CHILDHOOD

I *Estimation of IgG IgA and IgE Antibodies to Food Antigens in Children with Food Allergy and Atopic Dermatitis*

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ABSTRACT Dannaeus A Johansson S G O Foucard T and Ohman S (Department of Paediatrics the Blood Center and Department of Dermatology University Hospital Uppsala Sweden) Clinical and immunological aspects of food allergy in childhood Acta Paediatr Scand 66 31 1977.—Sixtynine children with case histories of food intolerance and 30 food tolerant children with atopic dermatitis have been investigated regarding serum IgE levels and IgE- IgG and IgA-antibodies to some common foods Children with food intolerance had significantly higher IgE levels and to a larger extent specific IgE antibodies to the tested allergens IgE antibodies to cow s milk were found in 71% of the children with histories of cow s milk allergy but occurred also in similar titers in 27% of milk tolerant children with other food allergies IgE antibodies to egg white occurred in 88% of egg allergies but low and moderate titers were also found in 17% of children without food intolerance However all children with high titers had symptoms of egg allergy IgE antibodies to the fish allergen were only found in fish allergic children while IgE antibodies to soy bean and green peas were found less consistently The level of serum IgA antibodies to milk was similar in both groups The IgG antibody titers to all tested food antigens seemed to parallel the IgE antibody titer to the same food It was not possible to correlate the IgG antibody titers to symptoms

KEY WORDS Food allergy atopic dermatitis

In children adverse reactions to ingested foods can be caused by a reaction between an ingested food allergen and its corresponding IgE antibody Therefore the quantification of serum IgE and IgE antibody to food allergens may be an important tool in the investigation of patients with food intolerance (8)

This study was done as an attempt to shed further light on the participation of IgE antibodies in relation to antibodies of other classes of immunoglobulins in children with symptoms suggestive of food intolerance History and symptoms were correlated to the serum IgE level and to the levels of IgE IgG and IgA antibodies to some common food allergens

MATERIALS

The clinical material consisted of 99 children 48 boys and 51 girls ranging in age from 3 months to 15 years

The patients were investigated at the University Hospital outpatient clinics and divided into two groups The criterion for admission to Group I was a clinical history of food intolerance based on the parents observations Specific foods were suspected as causative when the reactions were clearly related to food ingestion and when striking improvement followed food elimination Reported manifestations included eruption of eczema urticaria angiooedema circumoral rash or gastrointestinal symptoms such as vomiting diarrhoea and sometimes abdominal pains Asthma and anaphylactic shock had occurred in some patients Sixty three of the children in Group I had eczema varying from very slight to severe and in addition 21 had asthma and/or allergic rhinitis

Group II consisted of 30 children with atopic dermatitis without any obvious intolerance to foods In this group only one patient had asthma and four children had hay

Table 1 *Some of the most common food allergens suspected by the parents of 69 children with a history of food intolerance (Group I)*

Suspected food	No of subjects	%
Egg	35	51
Fish	23	33
Citrus	22	32
Chocolate	20	29
Tomatoe	18	26
Nuts	15	22
Cow s milk	7	10
Green peas	3	4
Soy bean	0	0

fever A standard questionnaire was used to obtain a history of the patients and their families All except four were seen by one of us (A D) In addition to a detailed history and physical examination venous blood samples were drawn from each patient After clotting sera were sparated and stored at −20°C until analyzed

The age distribution was similar in both groups as was the age at onset of eczema A family history of eczema asthma and allergic rhinitis was obtained with about the same frequency in Group I and II but the relatives of the children in Group II generally had more vague symptoms

The children of Group I often had multiple food hyper sensitivities (Table 1) It is to be noted that 50% of them reported intolerance even to foods not listed in the table but only single patients reacted to each of these foods

METHODS

The IgE concentrations were measured by a direct sand wich type of radio-immuno-sorbent technique PRIST (Ceska & Lundkvist 1972) (4)

IgE antibody activity was determined by the radio-allergosorbent test RAST (19) as described in detail (10) The results of RAST were expressed as parts per thou sand (‰) of a reference serum with 1‰ or more considered positive All tests were done in duplicate

IgG and IgA antibody activity was measured by a modified RAST technique using radiolabeled immunosorbent purified rabbit anti IgG and IgA The cpm activity on the disc was after subtraction of the cpm of a negative serum pool expressed in parts per thousand (‰) of a cow s milk reaginic serum An activity exceeding 5 of that in the reference serum was considered positive IgG anti bodies to a purified fish allergen DS22 were measured by a method based on the binding of IgG antibody to Sepharose coated staphylococcus protein A (11) as described in detail (6) One tenth ml of a serum sample diluted 1/10 was incubated with 0 5 ml Sepharose protein A (Seph pA) for 2 hours The ^{125}I labeled DS22 allergen representing approx 100 000 cpm was then added for a second incubation over night After washing the radio-activity bound to Seph pA was measured in a gamma counter and expressed in parts per thousand of a reference serum

Allergens used in the experiments

The allergen were coupled to the cyanogen bromide activated cellulose paper discs as described (10) The non specific binding of IgE was tested against a non reaginic serum having an IgE level of approx 5 000 U/ml and for all allergen found to be 1‰

For the preparation of 1 000 allergen coated discs the following allergen preparations were used respectively

1ml of commercial fresh evaporized skimmed (lipid concentration 0 05%) cow s milk

29 mg of an egg white extract ($E280^{1cm}_{1\%} = 14$) as described by Bleumink & Young (3)

5 ml of crude fish extract (Vitrum AB Stockholm) batch nr 8152 1/10 w/v

A soy bean extract from a commercial soy protein (soja protein HKC) with 90% soy protein according to the specification prepared by dissolving 3 g in 30 ml 0 15 M saline After centrifugation 2 ml of the supernatant was used

A green pea extract prepared by extraction of 700 g grounded commercial deep frozen green peas (Felix AB Sweden) in 250 ml distilled water The mixture was centrifuged and 0 4 ml of the supernatant was used

20 μg of a purified cod fish muscle allergen DS 22 (1) kindly provided by Dr K Aas Oslo and Dr H Bennich Biomedical Centre Uppsala was labeled with 2 mCi ^{125}I using the Chloramine T technique (9)

Skin prick tests were performed with the same extracts *A wheal size of 2 mm* or more greater than the control was considered positive

RESULTS

The serum IgE level varied from 15 to 3 000 U/ml (arithmetic mean 455 U/ml) in Group I and from 15 to 350 U/ml (arithmetic mean 104 U/ml) in Group II The highest IgE levels were found in children who had coexistent asthma and allergic rhinitis (Fig 1) The average number of positive RAST tests was highest in Group I but increased in both groups with the IgE level

Cow s milk allergy

Seven children in Group I had a case history suggesting cow s milk allergy (Table 2) After milk ingestion five of them (numbers 2 3 4 6 7) were reported to have vomiting and diarrhoea and one of them also urticaria (number 1) Four of them (numbers 2 3 4 5) had eruption of the eczema when milk was ingested

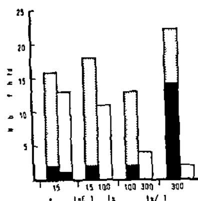


Fig 1 The distribution of serum IgE levels in 69 children with a history of food intolerance (Group I hatched bars) and in 30 eczema patients without symptoms indicative of food intolerance (Group II open bars) The number of patients with inhalant allergy is indicated by filled areas

Table 2 *The IgE levels and cow s milk antibody concentrations in serum of seven children with histories suggestive of cow s milk allergy*

The antibody values are expressed in parts per thousand of a standard

Case no	Age (mo)	IgE (units/ ml)	IgE antibodies (‰)	IgG anti bodies (%)	IgA antibodies (%)
1	16	157	9	970	195
2	20	3 000	2 570	182	1 000
3	9	48	5	230	340
4	58	193	3	200	1 000
5	3	26	7	40	240
6	10	10	<1	80	185
7	9	10	<1	87	290

The reactions were of the immediate type in all but 2 children (numbers 6 and 7) who had symptoms of delayed onset Five of the children had IgE antibodies to milk and raised serum IgE levels for their age Two patients (number 6 and 7 had normal serum IgE levels and no detectable IgE antibodies to milk All seven patients had antibodies to milk of the G and A immunoglobulin classes The highest levels were found in patients who also had IgE antibodies to milk (Table 2) IgE antibodies to milk could also be detected in children without obvious symptoms of cow s milk allergy (Table 3) Such antibodies were more frequent in Group I than in Group II No consistent covariation was found between the titers of IgE IgG and IgA antibodies Nor was there found any relationship between the antibody titers and clinical symptoms

Egg allergy

35/69 children (51 %) in Group I had histories suggesting egg allergy (Table 4) The symptoms varied from anaphylactic shock to a slight circumoral rash according to the parents observations 88 % of the egg hypersensitive children had detectable IgE antibodies to raw egg white and the titers seemed well correlated to the degree of clinical hypersensitivity All children with a titer exceeding 10‰ had symptoms of egg allergy

Four patients with a history of egg allergy had negative RAST tests A skin prick test with raw egg white was negative in these four children When challenged orally two children

Table 3 *Frequency of IgE IgG and IgA antibodies to cow s milk in children without obvious intolerance to milk in Groups I and II*

Antibody type	Group I %	Group I Mean titer ( )	Group I Range (‰)	Group II %	Group II Mean titer (‰)	Group II Range ( )
IgE	7	10	<1– 85	3	?	–
IgG	89	95	<1– 970	100	136	17– 450
IgA	100	380	5 –1 000	100	405	16–1 000

Table 4 *Frequency of IgE antibodies (positive RAST tests) and IgG antibodies to raw egg white*

35 children hypersensitive to egg (Group I) and 30 children without food intolerance (Group II)

	Group I			Group II		
Antibody type	%	Mean titer (‰)	Range (‰)	%	Mean titer (‰)	Range (‰)
IgE	88	275	<1-1 000	17	1 8	<1-2 0
IgG	94	98	<1- 600	67	38	<1-260

had circumoral rashes and two had no reaction

Low titers of IgE antibodies to raw egg white were also found in 21% of the children not sensitive to egg but to other foods These children also had positive skin tests with the egg white allergen

In Group II there were five patients (17%) who had IgE antibodies to raw egg white at even lower levels (Table 5) Only one of them had a positive skin prick test All but two of the egg allergic children had detectable amounts of IgG antibodies to egg white These two children had negative RAST tests The mean IgG antibody titer in Group I was significantly higher than in Group II $p<0\ 05$ (Table 4) The levels roughly parallelled the IgE antibody level but there were exceptions Five patients with very high IgE antibody titers to raw egg white had rather low titers of IgG antibodies The clinical symptoms of these children were not different from others

Fish allergy

24 children gave a history of fish allergy All types of reactions were represented but anaphylactic reactions in the respiratory and gastro intestinal tract dominated Quincke oedema urticaria and feeling of swelling and itching in the throat were also common symptoms

IgE antibodies were found in 16 children (67%) and only when the history indicated fish intolerance (Table 5)

All patients with a positive fish RAST had high serum IgE levels and symptoms of asthma or wheezy bronchitis Fish was generally not the only offending food All 16 children also had a varying degree of clinical egg hyper sensitivity and positive RAST to raw egg white Several patients also had intolerance to other foods

In children with a history of fish hypersensitivity IgG antibodies to fish as measured by RAST were demonstrable twice as often as in

Table 5 *Frequency of IgE antibodies (positive RAST tests) and IgG antibodies to isolated fish allergen DS 22*

Serum from 24 children with fish allergy according to history (Group I) and from 30 children without food intolerance (Group II)

	Group I			Group II		
Antibody	%	Mean titer (‰)	Range (‰)	%	Mean titer ( )	Range (‰)
IgE	67	395	<1-4 633	0	0	0
IgG	46	16	<1- 47	23	14	<1-155
IgG to DS 22	67	360	<1-2 315	0	0	0

Table 6 *Frequency of IgE antibodies (positive RAST tests) to soy bean and green peas and of IgG antibodies to soy bean*

Antibody type	Allergen	Group I %	Group I Mean titer (%)	Group I Range (%)	Group II %	Group II Mean titer (%)	Group II Range (%)
IgE	Soy bean	46	130	<1–1 683	23	3 8	<1–15
	Green peas	23	22	<1– 170	3	2 4	–
IgG	*Soy bean*	*58*	14	<1– 180	47	5	<1–2?

children with other kinds of food intolerance or in the children of Group II (Table 5) IgG antibodies to the pure fish allergen DS 22 as measured by the protein A technique showed a higher correlation to fish allergy and in Group I only those patients who had IgE anti bodies to fish had IgG antibodies to DS 22 The individual IgG antibody titer also roughly parallelled the titer of IgE antibody Fish sen sitive children with a negative RAST had more vague and less dramatic symptoms than the RAST positive children They also had normal serum IgE values and they neither had IgG antibodies to crude fish extract nor to DS 22 None of them had asthma or wheezy bron chitis

Soy bean and pea allergy

Although none of the children in this study was reported to be intolerant to soy bean 35 children were RAST positive against the soy bean allergen (Table 6) Skin prick tests per formed in 14 of those children were only posi tive in five cases who all had a RAST titer exceeding 15 %

IgG antibodies to soy bean were also a fre quent finding The highest titers (*mean 17%*) *and highest frequency of positive tests (70%)* were found in patients who had IgE antibodies to soy bean and the lowest titers were found in the children of Group II (Table 6)

Only three children gave a history sugges tive of pea allergy and they were all RAST positive to the pea allergen preparation with a mean titer of 29% However a further four teen children had demonstrable IgE antibodies of similar titers to the pea extract All children with IgE antibodies to the pea allergen also had a positive RAST test *to the soy bean prep*aration It was also found that fourteen out of 22 (67%) children positive in RAST to cow s *milk were positive in RAST to the* soy bean allergen

DISCUSSION

Most positive RAST tests were found in chil dren with high serum IgE levels

However in the youngest children with food intolerance it was often possible to find IgE antibodies to the tested foods even when the serum IgE was only *slightly elevated in* relation to age

Children with respiratory allergy in addition to eczema had *in agreement with* earlier re ports (20) high serum IgE levels including high RAST titers to the tested foods In these patients it was obvious that the titers did not always reflect the degree of clinical food hypersensitivity

Some patients reporting multiple food hy persensitivities had normal serum IgE concen trations and no detectable amount of IgE anti bodies *to the tested foods* Their *symptoms* were probably based on other immunological or non immunological mechanisms (2 14 16)

It is evident from this study and others (5) that IgE antibodies to cow s milk occur in

Table 4 *Frequency of IgE antibodies (positive RAST tests) and IgG antibodies to raw egg white*

35 children hypersensitive to egg (Group I) and 30 children without food intolerance (Group II)

	Group I			Group II		
Antibody type	%	Mean titer (%)	Range (%)	%	Mean titer (%)	Range (%)
IgE	88	275	<1-1 000	17	1 8	<1-2 0
IgG	94	98	<1- 600	67	38	<1-260

had circumoral rashes and two had no reaction

Low titers of IgE antibodies to raw egg white were also found in 21% of the children not sensitive to egg but to other foods These children also had positive skin tests with the egg white allergen

In Group II there were five patients (17%) who had IgE antibodies to raw egg white at even lower levels (Table 5) Only one of them had a positive skin prick test All but two of the egg allergic children had detectable amounts of IgG antibodies to egg white These two children had negative RAST tests The mean IgG antibody titer in Group I was significantly higher than in Group II $p<0.05$ (Table 4) The levels roughly parallelled the IgE antibody level but there were exceptions Five patients with very high IgE antibody titers to raw egg white had rather low titers of IgG antibodies The clinical symptoms of these children were not different from others

Fish allergy

24 children gave a history of fish allergy All types of reactions were represented but anaphylactic reactions in the respiratory and gastro-intestinal tract dominated Quincke oedema urticaria and feeling of swelling and itching in the throat were also common symptoms

IgE antibodies were found in 16 children (67%) and only when the history indicated fish intolerance (Table 5)

All patients with a positive fish RAST had high serum IgE levels and symptoms of asthma or wheezy bronchitis Fish was generally not the only offending food All 16 children also had a varying degree of clinical egg hypersensitivity and positive RAST to raw egg white *Several patients also had intolerance to* other foods

In children with a history of fish hypersensitivity IgG antibodies to fish as measured by RAST were demonstrable twice as often as in

Table 5 *Frequency of IgE antibodies (positive RAST tests) and IgG antibodies to isolated fish allergen DS 22*

Serum from 24 children with fish allergy according to history (Group I) and from 30 children without food intolerance (Group II)

	Group I			Group II		
Antibody	%	Mean titer (%)	Range (%)	%	Mean titer (%)	Range (%)
IgE	67	395	<1-4 633	0	0	0
IgG	46	16	<1- 47	23	14	<1-155
IgG to DS 22	67	360	<1-2 315	0	0	0

low RAST titers to specific allergens seem to be significant in children with clear cut symptoms of food allergy However later in child hood low levels of IgE antibodies to milk and egg seem to occur frequently even in children without obvious food intolerance (5) Whether this tolerance is caused by a diminished re sorption of allergen the occurrence of anti bodies with blocking capacity decreased sen sitivity for biological mediators in the target organs or some other mechanisms remains to be ascertained

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Submitted April 28 1976
Accepted Aug 24 1976

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rather high frequency even in children without obvious allergy to milk It is remarkable that in our study, only one of the milk intolerant children had a higher IgE antibody titer to milk than 16 milk tolerant children who had positive RAST tests to milk The clinical significance of a positive milk RAST seems to be highest in children 0–2 years of age

It seems reasonable that milk allergy in some patients can be triggered by an IgE antibody reaction especially when milk ingestion gives immediate symptoms from the skin and from the gastro intestinal tract The bad correlation between symptoms and IgE antibody titers is surprising Possible explanations are that the IgE milk antibody titer in serum does not reflect the true concentration of IgE antibodies in the mucous membranes and in the skin In addition to the allergen IgE antibody reaction several other factors may contribute to the symptomatology of cow s milk allergy

The presence of IgG antibodies to cow s milk is probably a physiological phenomenon in childhood (13) The titers often parallelled the IgE antibody titer to milk Even children on a milk free diet had in some cases high titers

IgA antibodies to cow s milk are also reported to be frequent in early childhood (13) In our study they seemed to occur parallel with the IgE antibodies It has been suggested that the maturation of the IgA system influences the liability of the child to produce IgE antibodies to food allergens (18) In our study the IgA antibody titer to milk could not be correlated either to IgE antibody titers to milk or to clinical symptoms

The clinical significance of both positive skin tests and RAST in egg white allergy is disputed (17) We found a good correlation between RAST titers and clinical symptoms No patient had a titer exceeding 10‰ without clinical symptoms of egg allergy Patients with low titers must be judged individually but generally no clinical hypersensitivity could be shown

IgG antibodies to egg-white were common in all children and parallelled the IgE antibody titer with few exceptions Many children showed increasing tolerance to egg and in these cases a high IgG antibody titer was a frequent finding Whether this high IgG antibody titer with a possible blocking capacity contributes to the increased tolerance to egg or is just a result of an increased allergenic load is not known

Fish allergy often gives systemic reactions and fish allergic children seemed to be less liable to outgrow their allergy during childhood In our study IgE antibodies to fish allergen were only demonstrated in the fish hypersensitive children and the titers were correlated to the degree of clinical sensitivity A rather high frequency of IgG antibodies to the crude fish allergen was found in children who were not sensitive to fish On the other hand only fish allergic children had IgG antibodies to the purified cod muscle allergen DS 22 This illustrates the need for purified allergens in the study of antibody patterns in atopic allergy

This study confirms reports that soy bean is a more potent immunogen than earlier believed (7) It is also worth noting that IgE antibodies to soy bean are a common finding in children with IgE antibodies to milk Several children had antibodies to both soy bean and green peas Whether this is caused by a higher immunoreactivity in these children or some cross reactivity on the basis of structural similarities between these allergens is not evident but the latter view is supported by a report that the legume family has allergenic determinants in common (15) Green peas were rarely suspected as an allergen and the significance of the high frequency of positive RAST tests to this allergen was difficult to evaluate

Food intolerance may be based on a multitude of different mechanisms Nevertheless the determination of the serum IgE level and IgE antibodies to certain food allergens can be helpful in evaluating the importance of type I reaginic allergy in individual cases In the first year of life even slightly raised IgE values indicate an atopic disposition (12) and even very

low RAST titers to specific allergens seem to be significant in children with clear-cut symptoms of food allergy However later in childhood low levels of IgE antibodies to milk and egg seem to occur frequently even in children without obvious food intolerance (5) Whether this tolerance is caused by a diminished resorption of allergen the occurrence of antibodies with blocking capacity decreased sensitivity for biological mediators in the target organs or some other mechanisms remains to be ascertained

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HIGH DENSITY LIPOPROTEIN CONCENTRATIONS IN NEWBORN INFANTS

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ABSTRACT Ginsburg B E and Zetterstrom R (Department of Paediatrics Karolinska Institute St Goran s Children s Hospital Stockholm Sweden) High density lipoprotein concentrations in newborn infants Acta Paediatr Scand 66 39 1977.—The lipoprotein pattern was analyzed by agarose gel electrophoresis in 19 newborn infants of varying gestational age The HDL concentration was determined by rocket immunoelectrophoresis in another 41 newborn infants Infants with a gestational age of <33 weeks had very low HDL concentrations compared to preterm infants with a gestational age of ≥33 weeks and term infants In the first 5–10 days after birth the HDL concentration increased markedly in preterm infants (gestational age <37 weeks) whereas it remained unchanged in term infants

KEY WORDS Newborn preterm lipoprotein pattern alfa lipoprotein HDL

Although HDL constitutes more than 50% of the total lipoproteins in the newborn infant this fraction has received very little attention In a study of the lipoprotein pattern in new born infants we observed that the alfa lipoprotein fraction (HDL) represented only about 10–15% of the total lipoproteins in newborn infants with a gestational age (gest age) below 33 weeks The concentration of HDL in serum obtained from newborn infants of varying gest age was determined and compared to the post natal age

MATERIALS AND METHODS

Materials

All infants were appropriate for gest age as judged by weight and length The lipoprotein pattern was analyzed on EDTA-plasma obtained from 11 term and 8 preterm infants The HDL concentration was determined on serum samples obtained from 39 term and 1? preterm infants In the preterm infants feeding was started within four hours after delivery by giving human breast milk in rapidly increasing amounts as devised by Davies (12) In infants with a gest age >36 weeks feeding was started 6–8 hours after delivery by giving 10 ml 5 5% glucose and after an additional 3–4 hours they were nursed by their mothers

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Lipoprotein electrophoresis Lipoprotein agarose gel electrophoresis was carried out according to the method described by Noble (11) The electrophoretogram was evaluated by densitometric scanning (5)

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The relation of HDL concentration to gest age was assessed with Sperman rang correlation test (3) and comparison of HDL levels in infants with a gest age of <33 weeks and ≥33 weeks respectivley was assessed with Student s *t* test

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Abbreviations HDL=high density lipoprotein S E = standard error S D =standard deviation

[1] HDL can be considered identical to alfa-lipoprotein when determined by this method



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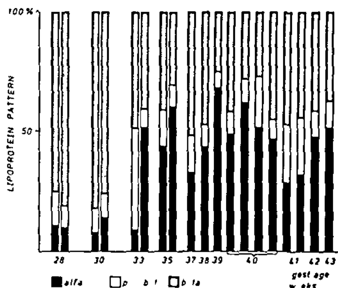


Fig 1 Plasma lipoprotein patterns in relation to gest age

Comparison of the postnatal increase in HDL concentration in preterm and term infants was assessed with the Wilcoxon sum of ranks test (4)

RESULTS

Lipoprotein pattern In term newborn infants alfa lipoprotein prebeta and beta lipoprotein accounted for 50 0±3 2% S E 11 0±1 8% and 37 3±2 5% of the total lipoproteins respectively In five term infants there was no difference between the lipoprotein pattern in cord blood and in blood collected from a peripheral vein nor did the pattern change within the first 9 hours Infants with a gest age of <33 weeks had very low alfa lipoprotein levels (13 9±3 5% S E) (Fig 1)

HDL concentration The HDL concentration in term newborn infants was 73 6±3 9% S E of the adult standard No difference was found between the HDL concentration in cord blood and in blood collected from a peripheral vein within 34 hours after delivery Infants with a gest age <33 weeks had lower HDL concentrations than infants with a gest age ≥33 weeks (p<0 01) (Fig 2) Whether there is a rather rapid rise in the 32nd–34th week of gestation or a continuous rise during a longer period can not be concluded from this study because of the small material During the first 5–10 days after delivery the HDL concentration rose markedly in preterm infants (gest age <37 weeks) but no consistent change was noted in term infants The difference was statistically significant (p<0 01) (Fig 3) After the initial rise no changes were noted during the following 3–5 weeks

DISCUSSION

There are many methodologic problems involved in performing lipoprotein electrophoresis during the neonatal period The colour density of the alfa region is increased by lipids and bilirubin bound to albumin The high levels of nonesterified fatty acids and bilirubin in the plasma of newborn infants reduce the reliability of the densitometric scan Immunologic quantitation of HDL is also associated with difficulties since HDL consists of several polypeptides These errors have to be taken into account in the evaluation of the results

This study confirms earlier observations in term newborn infants that alfa lipoprotein levels represent about 50% or more of the total lipoproteins (1 6 13) The HDL concentration in term newborn infants was found to be 73 6±3 9% of the adult value (S E) which is in good agreement with the value of 74 4± *15 1% (S D) reported by Davidsen (2)* In preterm infants with a gest age of <33 weeks

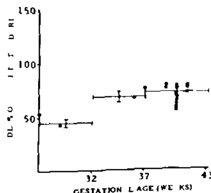


Fig 2 Serum HDL concentrations in relation to gest age The vertical bars indicate the standard error (S E) in three groups with gest age <33 33–37 and >37 weeks respectively

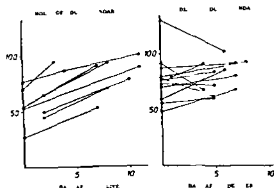


Fig 3 Changes in the serum HDL concentration after delivery in preterm (left) and term infants (right)

the HDL concentration was very low (45 1± 4 2%) Davidsen (2) also found low HDL values in infants with a gest age of <31 weeks (56 8% of adult values) the HDL values were highest in infants with a gest age of 35–38 weeks (84 0%) although they did not differ significantly from the values in infants with a gest age of 31–34 weeks and 39–42 weeks (78 6% and 74 4% respectively) In the study presented here the HDL concentration seemed to increase until the 33rd week of gestation but showed no further increase up to term It is tempting to speculate that the increase in the concentration of HDL which transports the majority of the serum phospholipids is related to a start of the biosynthesis of various phospholipids such as synthesis of surfactant factors (7 9) The major apo-HDL polypeptide apo-AI (apo-LP threonine) which represents 65–75% of the total HDL apoproteins is a co-factor to lecithin-chol esteryl acyl transferase (LCAT) (8)

Whether a low apo-AI concentration is the main cause for the low HDL level seen in infants with a gest age <33 weeks remains to be determined

The postnatal rise in HDL found in preterm infants markedly exceeded the prenatal alterations

ACKNOWLEDGEMENT

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Submitted May 19 1976
Accepted Aug 31 1976

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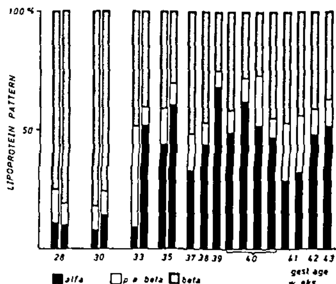


Fig 1 Plasma lipoprotein patterns in relation to gest age

Comparison of the postnatal increase in HDL concentration in preterm and term infants was assessed with the Wilcoxon sum of ranks test (4)

RESULTS

Lipoprotein pattern In term newborn infants alfa lipoprotein prebeta and beta lipoprotein accounted for 50 0±3 2% S E 11 0±1 8% and 37 3±2 5% of the total lipoproteins respectively In five term infants there was no difference between the lipoprotein pattern in cord blood and in blood collected from a peripheral vein nor did the pattern change within the first 9 hours Infants with a gest age of <33 weeks had very low alfa lipoprotein levels (13 9±3 5% S E) (Fig 1)

HDL concentration The HDL concentration in term newborn infants was 73 6±3 9% S E of the adult standard No difference was found between the HDL concentration in cord blood and in blood collected from a peripheral vein within 34 hours after delivery Infants with a gest age <33 weeks had lower HDL concentrations than infants with a gest age ≥33 weeks ($p<0\ 01$) (Fig 2) Whether there is a rather rapid rise in the 32nd–34th week of gestation or a continuous rise during a longer period can not be concluded from this study because of the small material During the first 5–10 days after delivery the HDL concentration rose markedly in preterm infants (gest age <37 weeks) but no consistent change was noted in term infants The difference was statistically significant ($p<0\ 01$) (Fig 3) After the initial rise no changes were noted during the following 3–5 weeks

DISCUSSION

There are many methodologic problems involved in performing lipoprotein electrophoresis during the neonatal period The colour density of the alfa region is increased by lipids and bilirubin bound to albumin The high levels of nonesterified fatty acids and bilirubin in the plasma of newborn infants reduce the reliability of the densitometric scan Immunologic quantitation of HDL is also associated with difficulties since HDL consists of several polypeptides These errors have to be taken into account in the evaluation of the results

This study confirms earlier observations in term newborn infants that alfa lipoprotein levels represent about 50% or more of the total lipoproteins (1 6 13) The HDL concentration in term newborn infants was found to be 73 6±3 9% of the adult value (S E) which is in good agreement with the value of 74 4± 15 1% (S D) reported by Davidsen (2) In preterm infants with a gest age of <33 weeks

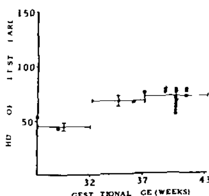


Fig 2 Serum HDL concentrations in relation to gest age The vertical bars indicate the standard error (S E) in three groups with gest age <33 33–37 and >37 weeks respectively

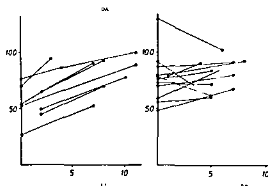


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Submitted May 19 1976
Accepted Aug 31 1976

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Acta Pædiatr Scand 66 43–48 1977

CONCENTRATIONS OF TRIGLYCFRIDES FREE FATTY ACIDS AND GLYCEROL IN CORD BLOOD OF NEWBORN INFANTS WITH A BIRTH WEIGHT OF ≤2 700 GRAMS

NIELS CHRISTIAN CHRISTENSEN

From the Departments of Obstetrics and Paediatrics Odense University Hospital Odense Denmark

ABSTRACT Christensen N C (Departments of Obstetrics and Paediatrics Odense University Hospital Odense Denmark) Concentrations of triglycerides free fatty acids and glycerol in cord blood of newborn infants with a birth weight of ≤2 700 grams Acta Paediatr Scand 66 43 1977.—Concentrations of triglycerides free fatty acids (FFA) and glycerol were measured in umbilical venous blood from 99 infants with a birth weight of between 1 100–2 700 g and a gestational age of 27–41 weeks Thirty infants were small for gestational age (SGA) 58 were appropriate (AGA) and 11 were of uncertain gestational age In AGA infants with a gestational age of ≤35 weeks FFA values were lower than in those with a gestational age of >35 weeks otherwise concentrations of triglycerides FFA and glycerol were independent of birth weight and gestational age in AGA infants In SGA infants higher FFA values were found compared with both AGA and term infants of normal birth weight Triglyceride values were higher in SGA than in AGA infants In SGA infants a significant positive correlation was found between gestational age and concentrations of both FFA and triglycerides No differences in FFA glycerol and triglyceride concentrations were seen between asphyxiated and non asphyxiated AGA infants

KEY WORDS Cord serum newborn low birth weight FFA glycerol triglycerides

Interest in the determinations of lipids in the cord blood has increased in recent years mainly in order to obtain an early diagnosis in children suffering from primary hyperlipoproteinemia Thus it appears possible to find children with primary hypercholesterolemia by measuring the concentration of either cholesterol (14) or very low density lipoprotein cholesterol (8) in the cord blood

In order to define more clearly these conditions it is necessary to understand the factors apart from the genetical that can influence the lipoprotein concentrations in cord blood This does not appear to have been studied sufficiently with regard to the triglyceride concentrations as the relatively few investigations published so far have given conflicting results

Sabata et al (11) studied the influence of asphyxia on the triglyceride concentration in cord blood and found the same values in children with slight and severe asphyxia and in children from a control group On the other hand Tsang et al (13) and Andersen & Friis Hansen (1) found that hypertriglyceridemia occurred more frequently in children with asphyxia than in those who had not

The triglyceride concentrations in cord blood from infants with a low birth weight has been studied by Fosbrooke & Wharton (5) who found that infants with low birth weight born at term had higher triglyceride values than mature infants of normal weight who again had higher values than premature infants Neither Brody & Carlson (2) nor Sabata et al (12) were able to demonstrate any difference between premature and mature infants by measuring the triglyceride concentrations

The present investigation was carried out in

Table 1 *Concentrations of triglycerides FFA and glycerol in umbilical venous blood*

All infants without asphyxia grouped according to birth weight Mean values for term infants with birth weight >3 000 g are inserted

Birth weight (g)	n	Triglycerides (mmol/l)		FFA (mmol/l)		Glycerol (mmol/l)	
		Mean	S D	Mean	S D	Mean	S D
≤1 500	3	0 47	0 28	0 19	0 07	0 07	0 01
1 501–2 000	15	0 43	0 14	0 20	0 12	0 06	0 04
2 001–2 500	31	0 58	0 27	0 27	0 13	0 07	0 04
2 501–2 700	12	0 49	0 23	0 23	0 10	0 07	0 02
Normal newborn infants (3)		0 52		0 24		0 07	

Only the difference between triglyceride values for the weight groups 1 501–2 000 and 2 001–2 500 is significant ($p<0$ 05 t test)
$n=37$

order to determine the concentration of triglycerides in cord blood from infants of low birth weight but of different gestational ages and with various perinatal complications including asphyxia The results were compared with the findings in a previous study of normal newborn infants (3)

There is a close metabolic relationship between free fatty acids (FFA) and triglycerides and an increase in the triglyceride concentration may have been preceded by an increased mobilization of FFA from adipose tissue providing the possibility of increased hepatic synthesis of triglycerides Therefore concentrations of FFA and glycerol have also been measured

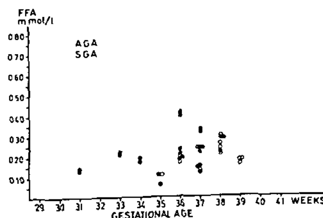


Fig 1 Relationship between cord blood FFA and gestational age

MATERIAL

The material includes 99 infants with a birth weight of ≤2 700 g and gestational ages of 27–41 completed weeks All children were born in the Department of Obstetrics Odense University Hospital The gestational age was calculated from menstrual data where these were reliable supplemented by clinical evaluation In 11 cases the gestational age could not be estimated with sufficient accuracy and these infants were excluded from the calculations as regards gestational age

Estimated on the basis of weight curves from this hospital (15) 30 infants were small for gestational age (≥2 S D below mean weight for gestational age) SGA infants while 58 were appropriate for gestational age AGA infants Mean weight and gestational age for SGA infants were 2 247 g and 38 2 weeks and for AGA infants 2 201 g and 35 0 weeks respectively The difference between the gestational ages was highly significant Twins and children born of mothers with chronic diseases such as diabetes mellitus were not included

Eighty seven of the women had uncomplicated pregnancies Among the others six had preeclampsia Thirteen of the infants were born by Caesarean section in 17 cases the birth was provoked using drugs and two infants were born by vacuum extraction

Asphyxia was evaluated from the Apgar score and arbitrarily divided into severe and slight asphyxia Slight asphyxia was defined by a 1 minute score of <7 and a 5 minute score ≥7 while severe asphyxia was present when both scores were <7 12 infants had slight and seven severe asphyxia

METHODS

Immediately following delivery blood was drawn from the umbilical vein Part of the sample was stabilized with EDTA Samples were centrifuged immediately or within 1 hour following storage at 4°C Analysis was performed on fresh centrifu[illegible]

Serum was analysed for triglycerides and glycerol (4) and plasma for FFA using the method of Laurell & Tibbling (9) as described previously (3)

RESULTS

The influence of birth weight gestational age and nutritional status on the concentrations of triglycerides glycerol and FFA was studied in 69 infants who did not suffer from asphyxia and showed no sign of disease during the first day of life 38 of the infants were AGA 24 were SGA and seven of uncertain gestational age

Table 1 shows triglyceride FFA and glycerol values for the infants grouped according to birth weight No differences between the various groups were found except for the significantly higher triglyceride values among infants with a birth weight of 2 001–2 500 g compared with those with a birth weight of 1 501–2 000 g Study of correlations between birth weight and concentrations of triglycerides glycerol and FFA respectively did not reveal any significant correlations in the whole group or when SGA and AGA infants were analysed separately

Relationships between gestational age and concentrations of FFA and triglycerides are shown in Figs 1 and 2 There was an increase in both the FFA and the triglyceride concentration after 35 weeks resulting in significant

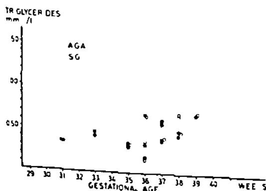


Fig Relationship between cord blood triglyceride values and gestational age

Table 2 *Coefficient of correlation (r) and levels of significance (p) between gestational age and concentrations of triglycerides and FFA in umbilical venous blood*

	r	*n*	*p*
All infants			
FFA versus gestational age	0 4410	62	<0 001
Triglycerides versus gestational age	0 39[illegible]	56	<0 01
AGA			
FFA versus gestational age	0 30[illegible]1	38	>0 05
Triglycerides versus gestational age	0 1697	36	>0 05
SGA			
FFA versus gestational age	0 4662	[illegible]4	<0 05
Triglycerides versus gestational age	0 5[illegible]8[illegible]	[illegible]0	<0 05

correlations between gestational age and concentrations of FFA and triglycerides (Table 2)

Inspection of the individual values in Figs 1 and 2 showed that the correlations between gestational age and FFA and triglyceride concentrations were caused mainly by the higher values in the SGA infants When SGA and AGA infants were analysed separately a significant correlation between gestational age and concentrations of triglycerides and FFA was found in the SGA infants only (Table 2) No significant correlation was found between gestational age and concentration of glycerol

As shown in Table 3 SGA infants had significantly higher triglyceride and FFA values than AGA infants whereas no difference was found in glycerol concentrations

Although there was no significant correlation between FFA concentration and gestational age it was found that AGA infants born before 36 weeks gestation had significantly lower FFA values than those born after 36 weeks gestation (Table 4) The mean values of triglycerides FFA and glycerol for AGA infants of more than 35 weeks gestation closely resemble those found in term infants of normal birth weight

Table 3 *Concentrations of triglycerides FFA and glycerol in umbilical venous blood from AGA and SGA infants*

	n	Triglycerides (mmol/l)		FFA (mmol/l)		Glycerol (mmol/l)	
		Mean	S D	Mean	S D	Mean	S D
AGA	36	0 47	0 19	0 21	0 08	0 07	0 04
SGA	20	0 61	0 29	0 30[b]	0 15	0 06	0 04
p values t test		<0 05		<0 01		>0 05	

n=38 [b] n=24

When SGA infants with a gestational age of 38–41 weeks were compared with term infants with a birth weight of >3 000 g (Table 1) significantly higher FFA values were found among the SGA infants (0 35 mmol/l versus 0 24 mmol/l t test p<0 01) The triglyceride values were also higher in SGA infants (0 68 mmol/l) but the difference was not significant as evaluated by the t test

A comparison of AGA and SGA infants of 35–38 weeks gestation did not reveal any significant differences between mean values of FFA triglyceride and glycerol concentrations although SGA infants had marginally higher FFA and triglyceride values (AGA/SGA infants FFA 25/29 mmol/l triglycerides 49/59 mmol/l)

An attempt to find a correlation between concentrations of triglycerides and FFA and the degree of intrauterine malnutrition (expressed as

$$\left(\frac{\text{birth weight} \times 100}{\text{mean birth weight for gestational age}}\right)$$

in SGA infants did not reveal any significant correlation

Eight babies with a mean birth weight of 2213 g and a mean gestational age of 37 3 weeks were delivered by Caesarean section and did not suffer from asphyxia Five of these were SGA Cord blood concentrations of triglycerides and FFA (0 54 mmol/l and 0 23 mmol/l) did not differ from the values obtained from infants born by spontaneous vaginal birth The concentration of glycerol was found to be lower for infants born by Caesarean section than for those born by vaginal birth (0 05 mmol/l versus 0 08 mmol/l p< 0 05)

Fourteen infants (10 AGA two SGA two of uncertain gestational age) with a mean birth weight of 2 314 g and a mean gestational age of 36 2 weeks were born by provoked labour using synticinon papaverin drop and with a normal Apgar score These infants had a mean triglyceride value that was slightly lower (0 42 mmol/l) than that found in infants born by spontaneous labour but the difference was not

Table 4 *Concentrations of triglycerides FFA and glycerol in umbilical venous blood from AGA infants grouped according to gestational age*

	n	Triglycerides(mmol/l)		FFA (mmol/l)		Glycerol (mmol/l)	
		Mean	S D	Mean	S D	Mean	S D
Gestational age ≤35 weeks	19	0 44	0 17	0 18	0 06	0 06	0 03
Gestational age >35 weeks	17	0 50	0 21	0 25	0 08	0 08	0 04
p values t test		>0 05		<0 01		>0 05	

significant nor did these infants differ from the others with respect to FFA (0 24 mmol/l) and glycerol (0 06 mmol/l)

Cord blood samples were obtained from five children whose mothers had suffered from preeclampsia Three of these children—two of whom were SGA—had a normal Apgar score and had moderately increased triglyceride values (mean 0 72 mmol/l)

Twelve infants suffered from slight asphyxia Seven of them were AGA and their mean values for FFA glycerol and triglycerides (0 18 0 08 and 0 43 mmol/l respectively) did not differ from those found in infants without asphyxia Four were SGA and had high triglyceride values (2 07 1 24 1 58 and 0 68 mmol/l respectively) and two had high FFA values (0 33 and 0 32 mmol/l)

Only seven infants all of whom had very low birth weight (mean 1486 g mean gestational age 31 2 weeks) had severe asphyxia Their mean triglyceride FFA and glycerol values (0 50 0 22 and 0 07 mmol/l respectively) did not differ from those found in infants without asphyxia

DISCUSSION

The main object of this study was to investigate whether the concentrations of FFA triglycerides and glycerol were influenced by birth weight and/or gestational age With respect to AGA infants it was found that apart from a slightly lower FFA concentration in babies with a gestational age of ≤35 weeks no such influence could be shown Cord blood triglyceride levels in preterm babies have been studied by Fosbrooke & Wharton (5) who found lower values in preterm (≤36 weeks) AGA infants than in term babies and by Sabata et al (12) who found similar values in premature (≤37 weeks) and in term babies It was not stated whether SGA babies were excluded from the latter study Previously reported FFA levels have shown similar values for term babies and preterm babies (12) or preterm AGA babies (6) Although it is difficult to compare these results it might be concluded that for AGA infants of a gestational age of >35 weeks FFA triglyceride and glycerol levels are independent of gestational age and birth weight The smaller premature babies might have slightly lower triglyceride and/or FFA levels

The results were different in the SGA babies Compared with AGA babies the SGA babies had higher FFA and triglyceride levels and an increase in FFA and triglyceride levels was seen with increasing gestational age Compared with term babies of normal birth weight SGA babies had higher FFA values but the difference between mean triglyceride levels was not statistically significant In the study by Fosbrooke & Wharton (5) higher triglyceride values were found in SGA babies compared with both preterm and term AGA babies These results as well as those of the present study show marked inter individual variations in triglyceride values This may well reflect that the SGA babies are an inhomogeneous group with regard to duration and cause of the intrauterine malnutrition Support for the assumption that low birth weight for gestation is a significant factor with regard to hypertriglyceridemia may be found in the study by Tsang et al (13) who found that six SGA babies all had hypertriglyceridemia and it is in agreement with the correlation found by Andersen & Friis Hansen (1) between hypertriglyceridemia and placental insufficiency

In a study comprising AGA SGA and term babies Harris (6) found similar FFA values in all three groups No obvious explanation of why his results are in contrast to those of the present study has been found

The present study does not confirm the observation by Tsang et al (13) and by Andersen & Friis Hansen (1) of a correlation between low Apgar score and other signs of fetal distress and hypertriglyceridemia Both these studies were mainly concerned with term infants of normal birth weight However no distinction was made between dysmature and mature babies in the asphyxiated groups In

Table 3 *Concentrations of triglycerides FFA and glycerol in umbilical venous blood from AGA and SGA infants*

	n	Triglycerides (mmol/l)		FFA (mmol/l)		Glycerol (mmol/l)	
		Mean	S D	Mean	S D	Mean	S D
AGA	36	0.47	0.19	0.21	0.08	0.07	0.04
SGA	20	0.61	0.29	0.30[b]	0.15	0.06	0.04
p values *t* test		<0.05		<0.01		>0.05	

n=38 [b] *n*=24

When SGA infants with a gestational age of 38–41 weeks were compared with term infants with a birth weight of >3 000 g (Table 1) significantly higher FFA values were found among the SGA infants (0.35 mmol/l versus 0.24 mmol/l, *t* test $p<0.01$) The triglyceride values were also higher in SGA infants (0.68 mmol/l) but the difference was not significant as evaluated by the *t* test

A comparison of AGA and SGA infants of 35–38 weeks gestation did not reveal any significant differences between mean values of FFA triglyceride and glycerol concentrations although SGA infants had marginally higher FFA and triglyceride values (AGA/SGA infants FFA 25/29 mmol/l triglycerides 49/59 mmol/l)

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Fourteen infants (10 AGA two SGA two of uncertain gestational age) with a mean birth weight of 2 314 g and a mean gestational age of 36.2 weeks were born by provoked labour using synticinon papaverin drop and with a normal Apgar score These infants had a mean triglyceride value that was slightly lower (0.42 mmol/l) than that found in infants born by spontaneous labour but the difference was not

Table 4 *Concentrations of triglycerides FFA and glycerol in umbilical venous blood from AGA infants grouped according to gestational age*

	n	Triglycerides (mmol/l)		FFA (mmol/l)		Glycerol (mmol/l)	
		Mean	S D	Mean	S D	Mean	S D
Gestational age ≤35 weeks	19	0.44	0.17	0.18	0.06	0.06	0.03
Gestational age >35 weeks	17	0.50	0.21	0.25	0.08	0.08	0.04
p values *t* test		>0.05		<0.01		>0.05	

Acta Pædiatr Scand 66 49–51 1977

ENDOCRINOLOGICAL INVESTIGATION OF PITUITARY CONADAL AXIS IN THALASSEMIA MAJOR

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ABSTRACT Anoussakis Ch Alexiou D Abatzis D and Bechrakis G (Endocrinological Department of the Aglaia Kyriakou Children s Hospital Athens Greece) Endocrinological investigation of pituitary gonadal axis in Thalassemia Major Acta Paediatr Scand 66 49 1977.—A study of the pituitary gonadal axis was undertaken in 18 male patients with thalassemia major and 41 normal males in order to define the cause of the sexual infantilism frequently seen in children affected by this illness The plasma testosterone level was measured before as well as after stimulation by human chorionic gonadotropin (HCG) Serum gonadotropin level was also determined during prepubertal pubertal and post pubertal age Significant findings were an insufficient secretion of gonadotropins as well as low testosterone level in patients of pubertal and post pubertal age A normal response of testes to HCG stimulation was also observed

KEY WORDS Sexual infantilism hemoglobinopathies testosterone gonadotropin

It is well known that patients with thalassemia major often show some complete sexual infantilism (2 3 4 6 7 9) However its cause remains until now undetermined The few reports on sexual immaturity of patients with thalassemia major were inconclusive about this point The present study must be considered as an attempt to elucidate the endocrine dysfunction responsible for the sexual infantilism of these patients

MATERIALS AND METHODS

Our material includes 18 male patients with thalassemia major (Table 1) and 41 normal males who served as controls The above material is divided into the following study groups

I Males prepubertal age

Patients In 9 patients 3 / to 11 / years old (mean age 6 / the plasma testosterone level was measured before and after human chorionic gonadotropin (HCG) stimulation of the testes The serum pituitary gonadotropins (FSH and LH) were also determined

Controls In 18 normal children / ₂ to 11 / ₂ years old (mean age 7 /) the plasma testosterone level was as above measured before and after stimulation of the testes In another 15 normal children 3 to 10 / ₂ years old (mean age 5³/ ₂) the serum pituitary gonadotropins (FSH and LH) were determined

II Males pubertal and postpubertal age

Patients The level of the serum pituitary gonadotropins was measured in 9 patients without any sign of puberty belonging to grade I of the classification of Tanner (13) 14³/ to 20 years old (mean age 16 / ₂) In 5 of the same patients the level of the plasma testosterone was also determined

Controls In 8 individuals 14 to 20 / years old (mean age 16 / ₂) with normal sexual maturation the level of the serum pituitary gonadotropins as well as the level of the plasma testosterone were measured

The HCG stimulation test (8) was performed as follows Three chorionic gonadotropin injections 1 500 IU each were made day after day Blood samples were obtained in the morning (10 to 1 a m) before the first injection and 4 hours after the last one After separation the plasma maintained at − 0°C

The determination of the plasma testosterone and the serum gonadotropins were made by radioimmunoassay (5)

RESULTS

I Males prepubertal age

As seen in Table 2 the patients mean plasma testosterone levels before and after HCG

agreement with the results of the present study Sabata et al (11) did not find any difference between triglyceride values for asphyxiated and non asphyxiated babies and the unchanged FFA values in asphyxia seem to be well established (6 7, 10 11) Further investigations are needed to elucidate the possible changes in triglyceride concentrations during asphyxia but from the present study it appears that one should take the nutritional status of the baby into account when evaluating the FFA and triglyceride concentrations in umbilical cord blood

ACKNOWLEDGEMENTS

This investigation was supported by a grant from Fonden for lægevidenskabelig Forskning m v ved Sygehusene på Fyn

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Submitted Sept 1 1975
Accepted Aug 29 1976

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Submitted Oct 10 1975
Accepted July 13 1976

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Table 1 *Clinical data of the studied patients*

No	Patients prepubertal age			Patients pubertal and postpubertal age		
	Age (y /mo)	Hb (g/100 ml)	Units[b] (transfused blood)	Age (y /mo)	Hb (g/100 ml)	Units[b] (transfused blood)
1	3/1	8 4	44	14/2	7 5	121
2	4/0	7 0	61	14/2	8 2	200
3	4/0	7 2	36	14/3	7 5	202
4	5/6	8 2	90	14/11	9 0	251
5	5/10	8 7	46	15/0	7 5	295
6	8/0	7 2	138	16/0	6 8	122
7	9/11	7 9	106	17/0	9 8	179
8	10/6	8 0	73	19/6	10 2	300[c]
9	11/9	7 5	189	20/0	8 2	178

Before last transfusion
[b] Total blood transfusion during patients life 1 unit of blood=250 ml packed red blood cells
[c] Splenectomy

stimulation, were 12 2±8 8 ng/100 ml and 148 6±50 5 ng/100 ml The mean values in the controls were 16 7±8 1 ng/100 ml and 166 0± 37 1 ng/100 ml respectively The observed differences between patients and controls before ($t=1$ 28 $p>0$ 05) and after HCG stimulation ($t=0$ 91 $p>0$ 05) were not statistically significant

Table 3 shows the values of the serum gonadotropins (FSH and LH) in the patients and the controls All the patients but one and the controls but two had plasma FSH values ≤0 4 ng/100 ml The differences here were also not significant

II *Males pubertal and postpubertal age*

Table 2 shows the plasma testosterone levels both in patients and controls of pubertal and postpubertal age It is obvious that the mean plasma testosterone level of the patients is significantly lower compared to the level of the controls ($t=14$ 4 $p<0$ 001) The mean serum gonadotropin levels are also significantly (FSH $t=5$ 48 $p<0$ 001 and LH $t=7$ 54 $p<0$ 001) lower in the patients than in the controls (Table 3) The above values compared to those found in the prepubertal age are significantly higher in the controls but without any considerable change in the patients

DISCUSSION

The testosterone level was found to be low during childhood However as shown by Saez & Bertrand (9) after stimulation by chorionic gonadotropin immature testes are able to re

Table 2 *Plasma testosterone level (ng/100 ml) in patients and controls before and after HCG stimulation of the testes*

Groups	Testosterone (ng/100 ml)	
	Before HCG (mean±S D)	After HCG (mean±S D)
Prepubertal age		
Controls	16 7±8 1	166±37 1
Patients	12 2±8 8	148 6±50 5
Pubertal and post pubertal age		
Controls	328 2±156 4	–
Patients	25±7 9	–

Table 3 *Serum gonadotropin (FSH and LH) levels (ng/100 ml) in patients and controls*

Groups	Gonadotropin (ng/100 ml)	
	FSH (mean±S D)	LH (mean±S D)
Prepubertal age		
Controls	≤0 4–0 5	0 64±0 3
Patients	≤0 4–0 6	0 57±0 21
Pubertal and post pubertal age		
Controls	1 32±0 43	2 18±0 47
Patients	0 57±0 47	0 84±0 20

Range of values

spond and secrete much more testosterone In our prepubertal patients as well as in the con trols a normal increase of the testosterone level after HCG stimulation has been ob served Lassman et al (7) also reported a nor mal post HCG response of the testes in a child with thalassemia major

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Submitted Oct 10 1975
Accepted July 13 1976

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LOW CONCENTRATION OF PLASMA AMINO ACIDS IN NEWBORN BABIES OF DIABETIC MOTHERS

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ABSTRACT Vejtorp M Pedersen J Klebe J G and Lund E (Medical Department T Bispebjerg Hospital and Diabetes Centre Department of Obstetrics and Gynaecology YB Rigshospitalet Copenhagen Denmark and the Danish Institute of Protein Chemistry Horsholm Denmark) Low concentration of plasma amino acids in newborn babies of diabetic mothers Acta Paediatr Scand 66 53 1977.—Plasma amino acid concentrations were measured in maternal peripheral vein and in umbilical vein and artery at birth (caesarean section) in 6 diabetic and 5 non-diabetic pregnancies The mean birth weight in the control group amounted to 3 9 kg as oversize of the foetus in three cases contributed to the indication for caesarean section The infants in the diabetic group consisted of small for gestational age babies (mean weight 2 8 kg) Free amino acid levels in the normal group and in diabetic maternal blood were in agreement with previous investigations No difference in amino acid concentrations in the maternal plasma was found but the concentrations of the umbilical vein plasma were significantly lower in the diabetic group Foetal hyperinsulinaemia may be a cause of the low amino acid concentrations Besides abnormalities of the placenta and maternal vascular complications increase and the mean birth weights decrease significantly through the White classes Conditions of impairment of placental transfer of amino acids may thus be present Characteristic features of the foetus may be consistent with the hypothesis as follows The foetus in diabetic pregnancy is in varying degree exposed to an oversupply of glucose hyperinsulinaemia imbalanced uptake and a slightly diminished supply of amino acids

KEY WORDS Diabetes mellitus newborn infant amino acids

The results of the few measurements of foetal and maternal concentrations of amino acids at birth in diabetic series have been conflicting (3 4 8 18)

We describe here a study of the concentrations of free amino acids in maternal peripheral venous plasma and in umbilical venous and arterial plasma at birth in a series of diabetic and non-diabetic pregnancies A hypothesis as regards the size at birth of infants of diabetic mothers (IDM) is advanced

PATIENTS AND METHODS

Patients

The material comprised a diabetic group of six mothers a non-diabetic group of five mothers and their newborn infants The diabetic series was nearly consecutive Two pregnancies belonged to White class B and four to class D (?0) The non-diabetic series was not consecutive The pregnancies were uneventful The urinary oestriol excretion was normal in the diabetic as well as in the control group

All of the infants were delivered by caesarean section The indications in the diabetic series (besides diabetes) were previous caesarean section (two) unsuccessful in

Table 1 *Clinical data*

Mean and range

Group	Maternal age (years)	Gestational age (weeks)	Birth weight (kg)	Birth length (cm)	Placental weight (g)
Diabetic	25 8	37 7	2 8	49 3	730
(*N*=6)	23–31	36–39	2 5–3 6	46–53	470–1 100
Non-diabetic	28 0	39 6	3 9	52 4	667
(*N*=5)	25–32	37–41	3 2–4 9	51–55	480– 970

duction (two) breech presentation (one) and foetal tachy cardia (one) In the control group the indications were contracted pelvis and previous section (one) contracted pelvis and oversized foetus (two) breech presentation and oversized foetus (one) and placenta previa (one) In all cases the anaesthesia was performed with nitrous oxi deoxygen after premedication with atropine and induction with enibomalsodium (Narcodorm) and succinylcholine The operations were done in the morning between 8 45 and 10 00 with one exception (at 2 50 p m in a diabetic) At 1 min Apgar scores were 4–10 in IDM and 2–9 in the control group at 5 min 9–10 in both groups No neonatal deaths occurred

Table 1 summarises maternal age gestational age birth weight birth length and placental weight

In the control group the mean birth weight amounted to 3 9 kg because presumed oversize of the foetus con tributed to the indication for caesarean section in three cases However the non-diabetic mother of the baby with a birth weight of 4 9 kg had a normal glucose tolerance test in the puerperium and also in a previous pregnancy and the mother of a baby with a birth weight of 4 4 kg was non glucosuric non-obese and without diabetes in the family The amino acid ratios F/M (concentrations in umbilical venous plasma/maternal plasma) of these two patients were in the middle of the values for the control group

Generally the birth weight of IDM is larger than that of normals (16) Consequently an IDM with a gestation time of 36–39 weeks and a birth weight of less than 2 8 kg is less than the 10 percentile and thus a small for gestational age baby (10) IDM constitutes a most inhomogenous population also inside one White class and the present series happened to comprise low weight babies with a mean birth weight of 2 8 kg or about 0 7 kg lower than usual found in our material (16) This is of importance in comparing the foetal amino acid concentrations in the present series with those of others

The mean gestational age was shorter in IDM but not significantly so Besides amino acid levels in normal pregnancies remain constant during the last trimester and into the postterm period in the mother and the foetus (23)

Methods

A few minutes before induction of anaesthesia periph eral venous blood from a maternal antecubital vein was taken in heparinized tubes From a segment of the cord insulated immediately after delivery blood was with drawn in heparinized syringes from an umbilical artery and the vein The time interval between induction of anaesthesia and withdrawal of the samples from the um bilical cord was about 5 min

After separation by centrifugation the proteins in 4 ml maternal plasma and 1 ml plasma from umbilical arterial and venous blood respectively were precipitated with picric acid which again was removed by passage through a resin column (Dowex 2–XB) The samples were then freeze-dried and stored at −18 C The maximal time interval between withdrawal of samples and freeze-drying was two hours

The samples were dissolved in sodium citrate buffer pH 2 2 and analyzed on Beckmann amino acid analyzers model 120 B and 121 Neutral and acidic amino acids were separated on ion exchange resin Type M 72 (Beckman Germany) using lithium citrate buffer as column eluant

Table 2 *Total plasma amino acids in maternal peripheral venous and umbilical venous plasma*

Median and range are given in mmol/l plasma

Group	Maternal plasma		Foetal plasma		Ratio F/M	
	Median	Range	Median	Range	Median	Range
Diabetic	1 68	1 41–2 17	2 09	1 90–2 80	1 3	1 01–1 72
Non-diabetic	1 60	1 27–1 71	2 84	2 55–3 19	1 9	1 78–2 07
	$p>0$ 10		$p<0$ 05		$p<0$ 01	

Median concentration in umbilical venous plasma/maternal plasma

Table 3 *Amino acid concentrations i maternal peripheral venous and umbilical venous plasma in the non diabetic group*

Median and range are given in μmol/l plasma $N=5$

Amino acid	Maternal plasma Median	Maternal plasma Range	Foetal plasma Median	Foetal plasma Range
Aspartic acid	5	3– 7	8	6– 9
Threonine	138	114–198	250	210–347
Serine	55	41– 93	138	108–164
Asparagine	20	17– 24	26	19– 28
Glutamine	248	175–256	407	256–435
Proline	89	75–112	124	101–173
Glutamic acid	73	17– 78	45	36– 51
Glycine	89	64–111	218	164–228
Alanine	201	174–262	270	241–341
Valine	118	94–114	199	152–229
Half-cystine	58	37– 83	69	63– 96
Methionine	3	3– 4	7	4– 10
Isoleucine	38	24– 42	56	42– 67
Leucine	68	47– 75	104	84–120
Tyrosine	27	19– 28	49	43– 61
Phenylalanine	35	27– 37	59	50– 74
Ornithine	21	16– 29	66	51–104
Lysine	128	83–151	320	267–480
Histidine	59	46– 76	98	92–130
Arginine	22	15– 28	75	54– 97

(1) The basic amino acids were separated on ion-exchange resin Type PA 35 (Beckmann USA) using sodium citrate buffer as column eluant (2) Standardized solutions of amino acids (Hamilton P AN and P B) were used for calibration after each preparation of ninhydrine and at time intervals of not more than one week The determinations were performed blindly and *in two* periods separated by a time interval of six months—due to change of location of The Danish Institute of Protein Chemistry In the first period samples from three controls and three patients with diabetes were examined No systematic difference between the determinations *performed* in the two periods was found

Twenty amino acids were determined in each sample of plasma In *one* diabetic pregnancy however the concentrations of six individual amino acids in umbilical artery blood were substituted for corresponding missing values in umb*ilical venous blood* in the calculation of the total amino acid concentration in the umbilical venous blood

The non parametric statistics were performed by the Mann-Whitney test the Wilcoxon test for pair differences and the Spearman coefficient of rank correlation The level of significance used was $p<0.05$

RESULTS

Total amino acids

In maternal peripheral venous blood the median concentration of total free amino acids in plasma was without significant difference between the diabetic and non-diabetic group In both groups of infants the median plasma concentration in umbilical venous blood was significantly higher than the maternal concentration but in IDM the median concentration was significantly lower than that in infants of non-diabetics ($p<0.05$) Also the median amino acid ratio F/M (median concentration umbilical venous plasma/maternal plasma) was significantly lower in the IDM ($p<0.01$ Table 2)

Individual amino acids

Free amino acid concentrations in maternal and umbilical venous plasma are shown for the non-diabetic group in Table 3 and for the diabetic group in Table 4 The individual amino acid concentrations in maternal plasma did not show any significant difference between the two groups In the groups taken together the concentrations of amino acids were significantly higher in umbilical venous plasma

Table 4 *Amino acid concentrations in maternal peripheral venous and umbilical venous plasma in the diabetic group*

Median and range are given in μmol/l plasma $N=6$

Amino acid	Maternal plasma Median	Maternal plasma Range	Foetal plasma Median	Foetal plasma Range
Aspartic acid	5	3– 7	6	5– 10
Threonine	174	115–223	221	184–302
Serine	69	53–110	109	98–156
Asparagine	30	15– 37	19	14– 29
Glutamine	218	147–391	213	158–492
Proline	94	75–150	115	81–161
Glutamic acid	55	33– 93	34	21– 41
Glycine	94	50–137	162	121–183
Alanine	192	158–284	243	192–284
Valine	142	97–194	174	145–228
Half-cystine	70	47–112	71	58– 79*
Methionine	3	3– 4	5	4– 15
Isoleucine	39	31– 60	44	37– 58
Leucine	64	60– 89	68	55– 98
Tyrosine	23	21– 36	34	24– 52*
Phenylalanine	32	28– 40	45	36– 70*
Ornithine	24	18– 42	63	50–101
Lysine	129	102–178	360	261–404
Histidine	69	43– 75	88	66–112
Arginine	25	13– 30	64	53– 95

* $N=5$

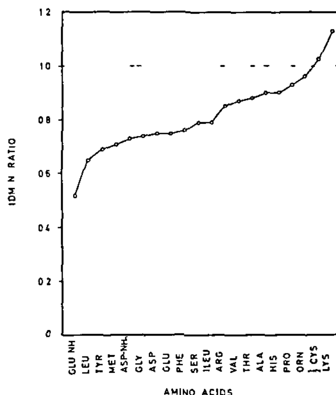


Fig 1 Ratios IDM/N (median concentration umbilical venous plasma in IDM/normal baby) of individual amino acids (× $N=5$)

than in maternal plasma with the exceptions that no significant difference in concentration was found for methionine aspartic acid asparagine half cystine and glutamine and that the concentration of glutamic acid was significantly higher in the maternal plasma (Tables 3 and 4) In general the individual amino acid concentration was lower in IDM than in normal infants but significantly so only for glutamic acid glycine and leucine When however an IDM/N ratio (median concentration in umbilical venous plasma at delivery in IDM/normal baby) for the individual amino acids was calculated the ratio was below 1 0 in no less than 18 out of 20 amino acids (Fig 1)

Also all of the F/M ratios for individual amino acids were lower in IDM than in the normal group but insignificantly so (Fig 2)

In the total material a tendency to higher concentrations of total and individual amino acids in umbilical venous than in umbilical artery plasma was found but a statistically significant difference was seen only for half cystine methionine and phenylalanine Contrarywise the concentration of glutamic acid was significantly higher in the umbilical artery plasma

No significant correlation was found either in the IDM or in the control group between birth weight and F/M ratio for total amino acids but the series is small

DISCUSSION

The concentrations found in this series of total free amino acids in non-diabetic and diabetic maternal blood and in normal infants and a F/M ratio of 1 9 in normals do not differ remarkably from those previously found (4 8 9,13) although as mentioned the mean birth weight amounted to 3 9 kg in the non diabetic group

A conspicuous result of this study was the demonstration of a significantly lower median concentration of free amino acids in umbilical venous plasma and a significantly lower F/M ratio in IDM compared with babies of non diabetic mothers

Previously the same result has been reported by Butterfield & O Brien (3) in one dia

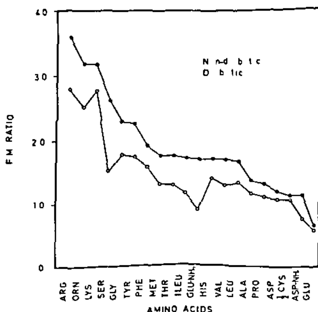


Fig 2 Ratios F/M (median concentration umbilical venous plasma/maternal plasma) of individual amino acids

betic and one prediabetic pregnancy and by Reisner et al (18) in 6 IDM although by extrapolation These findings however contrast with those of Ghadimi & Pecora (8) (three patients with advanced diabetes) and Cockburn et al (4) who found no difference in total maternal or foetal amino acid concentrations from normals Thus Cockburn et al calculated the F/M ratio to 1 6 in the normal as well as the diabetic series However their 9 IDM (White class B 1 C 5 D 2 F 1) were large for date babies with a mean weight of 3 8 kg or 1 kg more than that in the present series This difference in the two small series is not unnatural but should be kept in mind

It is of interest to point out that low F/M ratios also have been demonstrated in small for gestational age babies of mothers with a higher than normal maternal level (4 13) while in the present investigation the low ratio depended on a lower than normal foetal level

From previous investigations in human and more extensively in sheep and guinea pigs information has been gained concerning placental transfer of amino acids In general the transport is an active process operating against a concentration gradient The foetal supply depends on the active transport at the placental membrane the maternal blood flow and to a lesser extent the maternal concentration of amino acids The foetal pattern however seems to be related to foetal metabolism more than to the maternal supply (11 19 21 22)

The low foetal concentration of amino acids seen in IDM in this series may thus be due to an abnormal foetal amino acid metabolism and/or to a failure of the active transport at the placental membrane and/or to a decreased placental blood flow the consequence of which would be a lack of supply of amino acids to the foetus

Hyperinsulinaemia is a characteristic feature of IDM (16) Insulin promotes the transport of amino acids over cell membranes especially as regards the branched-chain amino acids valine leucine and isoleucine These amino acids are mainly if not exclusively metabolised in muscle A low concentration could be a consequence of hyperinsulinaemia In this series the ratio IDM/N was below 1 0 in 18 of 20 amino acids but only the concentrations of leucine glutamic acid and glycine was significantly lower in IDM However an insulin effect on the aminogram in IDM seems probable

The problem is whether there in addition to the effect of hyperinsulinaemia also is impairment of placental amino acid transfer resulting in foetal protein malnutrition and small size at birth From the present investigation of concentrations it is of course impossible to infer an impaired placental transfer Characteristic features of IDM may however point to a slight protein malnutrition

1) IDM are overweight predominately due to fat (7 15) but there is a significant decrease in the mean birth weight through the White classes (16)

2) Pathologic features of the diabetic placenta (16) and maternal vascular complications increase through the White classes

3) Although the total protein content of IDM seems normal (7 14) the serum protein concentration is decreased (5) and the development of ossification centers (17) muscles and bones (15) is retarded in relation to gestational age

4) The amino acid pattern in subclinical kwashiorkor (12) which according to the simplest concept is caused by a high carbohydrate supply and protein malnutrition has similarities with the pattern found in IDM (for details see (16))

The features of IDM may thus be consistent with oversupply of glucose hyperinsulinaemia imbalanced uptake and a slightly reduced supply of amino acids The low birth weight and low amino acid concentration in the present series as well as the normal amino acid concentration in the large babies in the series of Cockburn et al (4) may be an expression of this hypothesis

The hypothesis can be tested The concen

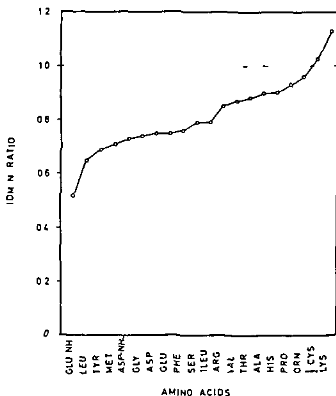


Fig 1 Ratios IDM/N (median concentration umbilical venous plasma in IDM/normal baby) of individual amino acids ($\bar{x}$ $N=5$)

than in maternal plasma with the exceptions that no significant difference in concentration was found for methionine aspartic acid asparagine half cystine and glutamine and that the concentration of glutamic acid was significantly higher in the maternal plasma (Tables 3 and 4) In general the individual amino acid concentration was lower in IDM than in normal infants but significantly so only for glutamic acid glycine and leucine When however an IDM/N ratio (median concentration in umbilical venous plasma at delivery in IDM/normal baby) for the individual amino acids was calculated the ratio was below 1 0 in no less than 18 out of 20 amino acids (Fig 1)

Also all of the F/M ratios for individual amino acids were lower in IDM than in the normal group but insignificantly so (Fig 2)

In the total material a tendency to higher concentrations of total and individual amino acids in umbilical venous than in umbilical artery plasma was found but a statistically significant difference was seen only for half cystine methionine and phenylalanine Contrarywise the concentration of glutamic acid was significantly higher in the umbilical artery plasma

No significant correlation was found either in the IDM or in the control group between birth weight and F/M ratio for total amino acids but the series is small

DISCUSSION

The concentrations found in this series of total free amino acids in non-diabetic and diabetic maternal blood and in normal infants and a F/M ratio of 1 9 in normals do not differ remarkably from those previously found (4,8 9 13) although as mentioned the mean birth weight amounted to 3 9 kg in the non diabetic group

A conspicuous result of this study was the demonstration of a significantly lower median concentration of free amino acids in umbilical venous plasma and a significantly lower F/M ratio in IDM compared with babies of non diabetic mothers

Previously the same result has been reported by Butterfield & O Brien (3) in one dia

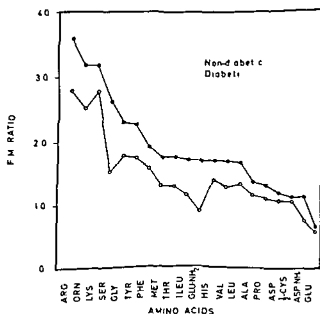


Fig 2 Ratios F/M (median concentration umbilical venous plasma/maternal plasma) of individual amino acids

Acta Pædiatr Scand 66 59–64 1977

EFFECT OF CONTINUOUS POSITIVE AIRWAY PRESSURE BREATHING ON CARDIORESPIRATORY FUNCTION IN INFANTS WITH RESPIRATORY DISTRESS SYNDROME

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KEY WORDS Preterm infants respiratory distress syndrome continuous airway pressure breathing

Continuous positive airway pressure (CPAP) is an established method of ventilatory assistance in severe respiratory distress syndrome (RDS) in newborn infants Since the first report by Gregory et al (12) on the treatment of RDS with CPAP a great number of clinical studies have taken place However little information is available regarding the effects of CPAP upon the cardiovascular system or on the ventilatory pattern and lung mechanics in infants with RDS

In the present paper a method of investigating some of these effects is described We have studied the following parameters of cardiorespiratory function in infants with RDS before and after the application of CPAP as well as during two or more levels of CPAP arterial pH oxygen and carbon dioxide tensions (P_{aO_2} and P_{aCO_2}) heart rate arterial blood pressure central venous pressure respiratory rate tidal volume minute ventilation and dynamic compliance

trations of amino acids should be lower in a group of White classes D+F than in one of White classes B+C infants with a significant difference in birth weight The placental trans fer of amino acids which may require whole blood determinations (6) might be experi mentally investigated

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Submitted April 12 1976
Accepted July 6 1976

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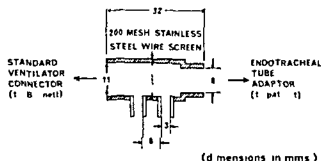


Fig 1 Design of pneumotachograph

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Oesophageal pressure was measured by means of a No 5 F G polyvinyl feeding tube the tip of which was covered by a latex balloon (length 5 cm diameter 8 mm thickness 0 014 mm) This was inserted through the mouth to the distal third of the oesophagus The volume of air in the balloon was 0 2–0 3 ml within which range pressure changes due to the elastic properties of the balloon were shown to be negligible (19) The pressure transducer (Bell and Howell type 4–422) which was used to measure oesophageal pressure was calibrated with a water manometer immediately after the study The difference in oesophageal pressure at end-expiration with and without CPAP was used to estimate the effect of CPAP on intrathoracic pressure

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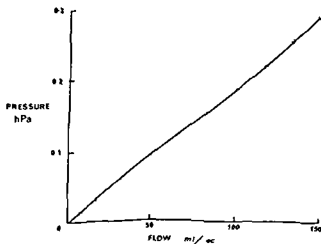


Fig 2 Linear relationship between pressure change and flow across pneumotachograph

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Blood pressure (hPa)			
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studied for a period of 0 3 kiloseconds at each given level of CPAP The direction of the change in CPAP was varied to avoid effects due to sequential changes During the last 60 s of each study period an arterial blood sample was obtained for the determination of pH P_{O_2} and P_{aCO_2} Continuous recordings of central venous pressure arterial blood pressure respiratory air flow tidal volume and oesophageal pressure were made on a 6 channel polygraph (type M19 Devices Ltd London England) but the values reported here are those recorded between 180–300 s at a given level of CPAP Heart rate was counted from the tidal volume recording Mean tidal volume and minute ventilation were calculated from all the breaths in the same 60 s

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$$PA_{O_2} = (P_B - P_{H_2O}) F_{IO_2} - PA_{CO_2}\left(F_{IO_2} + \frac{1-F_{IO_2}}{R}\right)$$

Where PA_{O_2} is alveolar oxygen tension F_{IO_2} is inspired oxygen concentration PA_{CO_2} is alveolar carbon dioxide tension assumed to be the same as arterial carbon dioxide tension R is respiratory quotient

RESULTS

The cardiorespiratory responses to CPAP are summarised in Table 2 and Fig 3 No consistent changes occurred in pH and P_{aCO_2} Arterial oxygenation improved in all infants on CPAP as shown by a significant increase in P_{aO_2} ($p<0\ 005$) and decrease in A aD_{O_2} ($p<0\ 01$) as asserted by the *t* test for pairs

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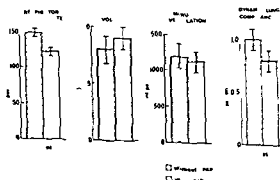


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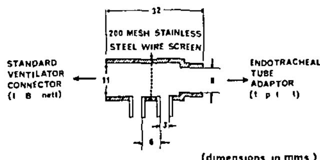


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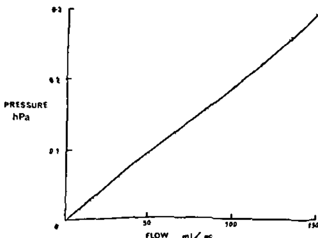


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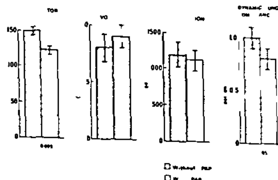


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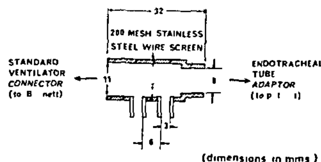


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Oesophageal pressure was measured by means of a No 5 F G polyvinyl feeding tube the tip of which was covered by a latex balloon (length 5 cm diameter 8 mm thickness 0 014 mm) This was inserted through the mouth to the distal third of the oesophagus The volume of air in the balloon was 0 2–0 3 ml within which range pressure changes due to the elastic properties of the balloon were shown to be negligible (19) The pressure transducer (Bell and Howell type 4–422) which was used to measure oesophageal pressure was calibrated with a water manometer immediately after the study The difference in oesophageal pressure at end-expiration with and without CPAP was used to estimate the effect of CPAP on intrathoracic pressure

The inspired oxygen concentration was maintained at the level used initially for clinical management and kept constant for the duration of the study The infant was

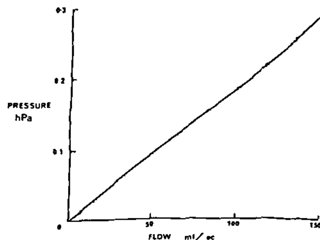


Fig 2 Linear relationship between pressure change and flow across pneumotachograph

percentage of CPAP may be transmitted to the intrathoracic space resulting in an increasing embarrassment of the circulation

We have found an increase in P_{aO_2} and a decrease in A aD$_{O_2}$ without a consistent change in P_{aCO_2} The increase in P_{aO_2} was thought to be a result of reducing right to left shunting of blood through the lung secondary to expansion of perfused but collapsed alveoli (12) However in the study of Bancalari et al (2) no correlation was found between the in crease in functional residual capacity and the change in P_{aO_2} These authors suggested that there is also a modification of extra pulmonary shunts secondary to a drop in pulmonary vas cular resistance They may be achieved by an increase in P_{aO_2} in lung volume or in vascular transmural pressure all of which are known to reduce pulmonary vascular resistance (5 16 24)

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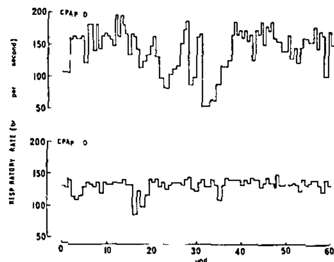


Fig 4. Breath by breath plot of respiratory rate.

for the 10 studies comparing values for the 60 s study period with and without CPAP. The results showed using analysis by the *f* test a significantly lower variance in instantaneous respiratory rate with CPAP in 7 of the 10 studies. A lower variance in instantaneous tidal volume with CPAP occurred in 9, 6 of which reached statistical significance.

DISCUSSION

The effects of an increase in transpulmonary pressure on the cardiovascular system *in the* newborn infant have not been adequately studied. Previous investigations were done mainly in adults with *normally compliant* lungs. When the airway pressure was increased, tachycardia, hypotension, a decrease in stroke volume and cardiac *output* were demonstrated in both human and animal studies (4, 8, 15, 20). In contrast little change in cardiac output was observed when 5 hectopascals was applied during expiration to the lungs of patients with pneumonia and "shock lungs" (18). The circulatory changes in adult studies were generally demonstrated at pressures greater than those employed for neonatal respiratory diseases.

No measurements of cardiac output or venous return with the use of CPAP in human infants suffering from RDS have been reported. It was assumed by Gregory et al. (12) that because the rise in oesophageal pressure with CPAP was only 20% of the pressure applied to the airway, the circulation was *probably not* appreciably compromised. Clinical observations may even suggest that infants with RDS on CPAP have an improvement of cardiovascular function coincidental with the improvement of oxygenation (6). In common with other clinical studies in which blood pressure was monitored *in some of the infants* studied, we found no significant changes in mean blood pressure with CPAP (3, 21, 23, 26). Although no obvious clinical deterioration was observed in any of our infants, the pulse pressure decreased and the central pressure increased with CPAP. Since the venous return *to the right atrium depends on the difference* in pressure between intrathoracic and extrathoracic veins, the application of CPAP can on a theoretical basis impede venous return (6, 17). It is therefore important that the use of *CPAP* in *RDS* must be approached with caution, particularly in situations of hypovolaemia and impaired cardiac output. Monitoring of arterial blood pressure and central venous pressure may be important at a time when the infant with RDS improves and the lungs become more compliant, when a greater

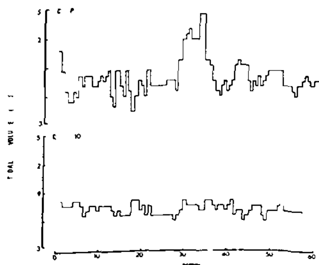


Fig 5. Breath by breath plot of tidal volume.

percentage of CPAP may be transmitted to the intrathoracic space resulting in an increasing embarrassment of the circulation

We have found an increase in P_{aO_2} and a decrease in $A\text{-}aD_{O_2}$ without a consistent change in P_{aCO_2}. The increase in P_{aO_2} was thought to be a result of reducing right to left shunting of blood through the lung secondary to expansion of perfused but collapsed alveoli (12) However in the study of Bancalari et al (2) no correlation was found between the increase in functional residual capacity and the change in P_{aO_2}. These authors suggested that there is also a modification of extra pulmonary shunts secondary to a drop in pulmonary vascular resistance They may be achieved by an increase in P_{O_2} in lung volume or in vascular transmural pressure all of which are known to reduce pulmonary vascular resistance (5 16 24)

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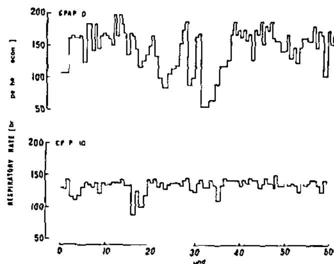


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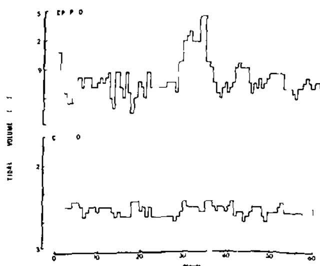


Fig 5 Breath by breath plot of tidal volume

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Submitted Febr 18 1976
Accepted July 1 1976

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Acta Pædiatr Scand 66 65–72 1977

SEASONAL INCIDENCE OF SOME CONGENITAL MALFORMATIONS IN THE CENTRAL NERVOUS SYSTEM IN SWEDEN 1965–1972

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ABSTRACT Sandahl B (Department of Obstetrics and Gynecology Malmo General Hospital Malmo and Department of Embryology University of Lund Sweden) Seasonal incidence of some congenital malformations in the central nervous system in Sweden 1965–1972 Acta Paediatr Scand 66 65 1977.—Seasonal trends of some congenital CNS malformations in infants born in Sweden during the years 1965 to 1972 were investigated The study is based on reports to the Swedish Register of Congenital Malformations statistics from the National Board of Health and records from Malmo General Hospital and the Hospital of Lund The malformations analysed in this study are anencephaly spina bifida aperta (with or without hydrocephalus) and isolated hydrocephalus Variations in incidence of months of birth and last menstrual period (LMP) were tested statistically in three different ways standard χ test for heterogeneity between recorded numbers of infants each calender month Edwards method and a squared sinus function all with or without correction for variations in general monthly birth rates Statistical significant seasonal variations were found for anencephaly with an LMP maximum in March and for spina bifida aperta with a maximum in July Factors which can explain such seasonality are briefly discussed

KEY WORDS Abnormalities anencephaly spina bifida hydrocephalus seasons

Many studies have been published on seasonal variations in malformation rates A critical review on early work in this field was given by Bailar & Gurian (1) The most wellknown example of seasonality in malformation rates is that found for anencephaly (2) and meningocele However there appear to be marked secular changes and also geographic differences in seasonality for this group of malformations (Table 1)

The present paper reports on an analysis of seasonality in some CNS malformation rates reported to the Swedish Register of Congenital Malformations supplemented with hospital data

MATERIAL AND METHODS

The following sources of material were used

1 The Swedish Register of Congenital Malformations Since 1965 infants born with malformations are reported from hospitals where the infants are examined by a paediatrician after birth (50 hospitals in 1965 60 in 197?) These hospitals are scattered over the greater part of the country and represent appr 65% in 1965 and 80% in 197 of the total number of births in Sweden The only excluded region is the Uppsala region (see below) This register has supplied information on the number of infants born each month with a certain malformation diagnosis Reports of such infants are divided into two groups 1) infants with only one reported malformation 2) infants with two or more simultaneous malformations For this study infants registered with only one malformation were analysed except for those with anencephaly where that diagnosis obviously dominates

2 National Board of Health collected detailed In patient statistics from hospitals in the Uppsala region 1964–1968 (3) This region was not included in the Register of Malformations until 1973 why data from the Swedish Register of Congenital Malformations were supplemented with informations from this source This source also provided information concerning gestational length of pregnancies resulting in infants with congenital malformations in the central nervous system (4)

3 Records from the Malmö General Hospital and the

Table 1 *A summary of some of the literature dealing with anencephaly concerning seasonality in incidence month of highest incidence correction for variation in gestational length and monthly birth variation place of study and total number of cases*

Given peak month is month of birth For references see Sandahl (10)

			Correction made for			
Authors	Seasonality	Peak month	Variation in gestational length	Monthly birth variation	Place and years	No of cases
Beolchini & Bailo 1965	Yes	Winter	Yes	Yes	Milano Italy 1937–64	77
Damyanow & Dutz 1971	Yes	Dec	No	No	Shiraz Iran 1966–70	23
Edwards 1958	Yes	Winter	No	Yes	Scotland 1939–56	
Edwards 1961	Yes	Jan	No	Yes	Scotland 1945–56	366
Elwood 1970	Yes	Oct	No	Yes	Belfast N Ireland 1950–66	584
Elwood & MacKenzie 1971	Yes	Winter	No	No	Scotland 1956–66	1 667
Leck & Record 1966	Yes	Nov	Yes	Yes	Scotland 1940–65	1 052
McKeown & Record 1951	Yes	Winter	Yes	Yes	Scotland 1939–46	1 916
Record 1961	Yes	Nov–Jan	No	Yes	Scotland 1949–58	2 671
Slater et al 1964	Yes	Winter	No	No	United Kingdom and Eire 1954–60	739
Tunte 1968	Yes	Winter	No	No	Munster Germany 1950–61	638
Cassady 1969	No		Yes	Yes	Alabama USA 1961–66	367
Czeizel & Révész 1970	No		No	Yes	Hungary 1963–67	360
Elwood & Nevin 1973	No		No	Yes	Belfast N Ireland 1964–68	151
Frezal et al 1964	No		No	Yes	France 1945–55	288
Halevi 1967	No		No	Yes	Israel 1959–60	55
Hewitt 1962	No		No	Yes	New York USA 1957–59	39
Laurence et al 1968	No		Yes	Yes	South Wales 1956–59	351
MacMahon et al 1953	No		Yes	Yes	Rhode Island USA 1936–52	376
Milham 1963	No		No	No	New York USA 1960	135
Roberts et al 1972	No		Yes	Yes	South Wales 1964–66	253
Smilkstein 1962	No		No	No	Los Angeles USA 1948–58	48
Wehrung & Hay 1970	No		No	Yes	USA 1962–65	2 066
Westerlund 1969	No		No	No	Norway 1951–65	159

Hospital of Lund (Departments of Gynaecology and Obstetrics of Pathology and Neurosurgery) These records were searched for infants born at the two hospitals during the period 1960–1973 with anencephaly spina bifida aperta or hydrocephalus Information on pregnancy length in these pregnancies was retrieved During the period 1965–1972 a number of cases were found that were not reported to the Register of Malformations—as shown by Källén & Winberg (5) these two hospitals showed a marked underreporting especially for anencephalic infants The Register material was supplemented with these cases

4 A prospective study performed in Malmo during 1963–1964 comprising appr 6400 pregnancies (6) Date of last menstrual period (LMP) and date of birth was known for all pregnancies ending with the birth of a normal infant and these data were used for estimating pregnancy length distribution (cf 7)

5 Official statistics of Sweden This source supplied information on the total number of births each calendar month for the period 1965–1972

Malformations Studied

A *Anencephaly* All infants born 1965–1972 with anencephaly reported to the Register of Congenital Malformations or to the In patient statistics from hospitals in the Uppsala region and such infants identified from hospital records in Malmo or Lund were used Only from this group were infants with more than one major malformation accepted

B *Spina bifida aperta* All infants reported to the Register of Congenital Malformations with meningocele myelomeningocele or myeloschisis were used if no other simultaneous malformation was reported the only exceptions being talipes or hydrocephalus These two malformations are not registered as separate entities in the Register when central nervous system dysraphism is present Infants with both anencephaly and spina bifida aperta were classified as anencephalic only

C *Hydrocephalus* Infants with hydrocephalus and without any other known malformation were accepted The definition of hydrocephalus which leads to the primary report is a head circumference more than 38 cm

Table 2 *Recorded monthly distribution of births of infants with some CNS malformations*
Swedish Register of Malformations 1965–1972 supplemented with some other material (see text)

	Total no	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Anencephaly	296	27	20	8	35	26	12	26	15	20	33	28	31
Spina bifida aperta	263	18	26	21	17	32	25	23	22	26	17	17	19
Hydrocephalus	90	9	1	6	4	8	7	6	9	10	6	9	4

where no obvious explanation exists in the form of massive scalp bleeding or oedema. In dubious cases the report is accepted only after follow up. Note that hydrocephalus secondary to spina bifida aperta is only classified under the latter diagnosis.

Statistical Methods

The actual numbers of malformed infants identified each calendar month were compared by three different methods in order to discover whether random variations could explain recorded differences.

χ² test

Standard χ² tests for heterogeneity were run between recorded numbers of infants each calendar month. They are based on 11 d.f. Such tests were made both on the number of infants born each month and on the number of pregnancies begun each month (LMP dates). When corrections for changes in monthly pregnancy rates were made, χ² tests (also at 11 d.f.) were performed between the found values and the expected values at no monthly differences in malformation incidence (null hypothesis).

Edwards' method

Edwards (8) described a method for analysing seasonal changes in, for instance, malformation rates. It is much superior to the simple χ² test as it utilizes the support given by adjoining months, i.e. seasonal *trend* in the materials. Principally the method consists of fitting a sinus curve to the recorded differences between found and expected number of malformed infants each calendar month after normal transformation. This method was applied on raw monthly data and on data after correction for pregnancy length and pregnancy rates. The χ² values obtained in this method are based on d.f. The method has been criticized by Hewitt et al. (9) as it can give significant results for data that do not show a real rhythmicity.

Suggested method

A drawback with Edwards' method is that it supposes a sinus curve as a reasonable mathematical model for the recorded fluctuations, i.e. a symmetrical curve with six months of increase and six months of decrease and with maximum and minimum incidence of the same magnitude. A modification was therefore tried which allows for asymmetrical periodic change during the year. As a possible mathematical model the following equation was used:

$$Y = \alpha \sin x + \beta \cos x$$

where x is the number of the calendar month expressed in 1/12 of 360 (= one year) and α and β are coefficients. The beginning of the cyclic function—the change from low to high values—is found empirically. This function is fitted to variates made up of the differences between found and expected numbers transformed to normality by the $2\sqrt{\ }$ method. The variates will thus be $Y = 2(\sqrt{F} - \sqrt{E})$ where F is the observed number—sometimes after corrections for e.g. pregnancy length—and E is the expected values under the hypothesis of constant malformation incidence during the year (null hypothesis). As only whole months are used, the sin x values will be the following: 0, 0.5, 0.866, 1.0, 0.866, 0.5, 0, −0.5, −0.866, −1.0, −0.866 and −0.5. By using the least squares method, the following estimates of α and β can be made:

$$\alpha = \frac{1}{6}\Sigma(Y \sin x)$$

$$\beta = \frac{}{9}\Sigma(Y \sin x)$$

and the sum of squares around the function so defined is

$$\Sigma d^2 = \Sigma Y^2 - \frac{1}{6}[\Sigma(Y \sin x)]^2 - \frac{}{9}[\Sigma(Y \sin x)]^2 \quad \text{based on 9 d.f.}$$

If seasonality is disregarded, the sum of squares is

$$\Sigma d_0^2 = \Sigma[2(\sqrt{F} - \sqrt{E})]^2 \quad \text{based on 11 d.f.}$$

The difference $\Sigma d_0^2 - \Sigma d^2$, based on ? d.f., is the sum of squares eliminated by the squared sinus function and it can be evaluated with an F test against the error variance around the function, i.e.

$$F_{2,9} = \frac{(\Sigma d_0^2 - \Sigma d^2)/2}{(\Sigma d^2)/9}$$

RESULTS

Table 2 presents the raw material, namely the number of infants born each month with the different malformations discussed. It also gives the total number of infants in each category. The smallest group consists of only 90 infants with isolated hydrocephalus. However, 90 is sufficient to give expected numbers of infants each month above 5 in all χ² tests performed.

Table 3 *Seasonality of normal births (according to Sandahl 1974) Per cent of all births in Sweden 1965–1972*

Month	%	Month	%
Jan	8 20	July	8 15
Febr	8 07	Aug	7 94
March	9 64	Sept	8 07
April	9 72	Oct	7 96
May	9 23	Nov	7 28
June	8 28	Dec	7 45

Seasonality can be analysed in different ways Months of birth or months of last menstrual period (LMP) can be compared The two methods will give different results if the length of pregnancy differs between normal infants and those with the malformation in question Seasonality can also be studied with or without considering monthly fluctuation in frequency of normal births

I *Seasonality according to month of birth*

The monthly distribution of births of infants with different malformations was studied with all three statistical methods χ^2 for heterogeneity between months Edwards method and the squared sinus function This was done on the uncorrected material and on the material corrected for the monthly fluctuation of normal births as recorded in an earlier paper (7) and shown in Table 3 The χ^2 values for heterogeneity in the column for corrected values in Table 4 is thus performed with the found number each month and the expected number that month presuming a constant incidence throughout the year (for January the expected number of a malformation with a total number of N will be 0 0820 N)

When Edwards method is used the found number a certain month was increased with a factor compensating for the uneven distribution of normal births (for January this factor will be 8 33/8 20=1 02)

Statistically significant results were obtained for anencephaly only

Anencephaly shows a probably significant variation in the distribution according to the χ^2-test for both uncorrected and corrected values, a seasonality can be detected also with Edwards method, but only for corrected values whereas the squared sinus method gives no support for a rhythmic seasonality

Fig 1 A shows the actual distribution of births at anencephaly and the deviations ($2[\sqrt{F}-\sqrt{E}]$) used for graph fitting

Either *spina bifida aperta* or *hydrocephalus* show any seasonality according to month of birth

II *Seasonality according to LMP*

It is possible that a seasonality can exist for a malformation when it is referred to the time of

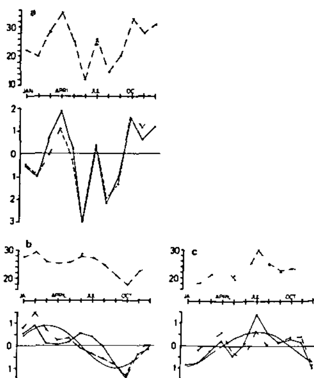


Fig 1 Monthly distribution of births (MB) and month of LMP of malformed infants Upper graph shows actual numbers of malformed infants born each month (dots and dashes) and expected numbers (dots) at a constant malformation rate but with consideration given to monthly variations in total birth rate Lower graph shows recorded deviations between found and expected numbers transformed to normality by the $2\sqrt{N}$ method Whole line is not corrected but dashed line is corrected for monthly variations in total birth rate Squared sinus functions are shown when significant (*a*) Anencephaly (MB) (*b*) anencephaly (LMP) (*c*) spina bifida aperta (LMP)

Table 4 *Seasonality according to monthly number of births (MB) and LMP with or without correction for variability in monthly birth rates of normal pregnancies*

Diagnosis	χ^2 for heterogeneity d f =11		Edwards model χ^2 d f =2		Squared sinus function			
					No corr		Corr	
	No corr	Corr	No corr	Corr	$F_{2,9}$	Month	$F_{\cdot 9}$	Month
Anencephaly								
MB	22 2	2? 7	4 0	7 1*	1 9	Oct	1 9	Oct
LMP	4 6	6 8	2 5	5 6	4 4	Dec	17 0	Dec
Spina bifida aperta								
MB	11 1	10 5	3 5	1 6	2 1	March	1 0	April
LMP	5 5	2 9	3 3	0 4	6 5	April	1 4	April
Hydrocephalus								
MB	8 7	10 9	0 3	1 4	<1	Aug	1 1	Aug
LMP	5 4	7 1	0 3	1 1	<1	Oct	2 0	Oct

Month of origin of the function i e sin x=0 changing from negative to positive
0 05>P>0 01 P<0 001

conception but that the seasonality becomes hidden by marked differences in gestational length for infants with the malformation In order to investigate this possibility the monthly distribution of LMP was studied instead The gestational length was not known for most infants as the date of LMP is not reported to the Register of Congenital Malformations The LMP distribution was therefore calculated as follows

For normal infants the pregnancy length was known for a sample consisting of 5606 infants (Table 3 cf Sandahl 7) From those values the distribution according to month of LMP for normal infants could be estimated For infants with CNS malformation samples with known gestational length were used to estimate the distribution according to LMP of the material from the Register of Congenital Malformations These samples were ascertained from records from Malmo and Lund hospitals and from the ' In patient statistics from the hospitals in the Uppsala region Table 5 shows the distribution of these births according to gestational age From these data the monthly distribution according to LMP was extrapolated for the material from the Register of Congenital Malformations This presupposes that the samples used for estimating pregnancy length and that contained in the Register of Congenital Malformations do not differ with respect to gestational length distribution which appears to be a reasonable supposition

Table 4 gives the values for χ^2 tests for heterogeneity χ^2 at Edwards model and F

Table 5 *Distribution of gestational age in weeks*

For normal infants each age class is given as % of all such births with its error For each type of malformation the actually found numbers (F) and those expected from the normal material (E) are given

Gestational age in weeks	Normal N=5606	Anencephaly N=110		Spina bifida aperta N=75		Hydrocephalus N=111	
		F	E	F	E	F	E
≤33	0 82±0 1?	18	0 9	1	0 6	1	0 9
34–37	6 19±0 3?	33	6 8	6	4 6	15	6 9
38–4	89 17±0 4?	28	98 1	61	66 9	90	99 0
≥43	3 8?±0 6	31	4 ?	7	2 9	5	4 ?

Table 6 *Variance analysis comparing the squared sinus functions estimating LMP incidence of anencephaly and spina bifida aperta after correction for monthly birth rate fluctuations*
Month of origin is the month where sinus $x=0$ and changes sign from negative to positive

Source of variation	Month of origin	χ and β	Sum of res squares	d f	Variance
Within both malformations	Common	Common	5 5496	21	
Within anencephaly	Own	Own	1 4060	9	0 1562
Within spina bifida	Own	Own	1 3640	9	0 1516
Within both malf	Own	Own	2 7700	18	0 1539
Between malf			2 7796	3	0 9265
Within both malf	Common	Own	3 9965	19	0 2103
Between malf			1 5531	2	0 7766
Within both malf	Own	Common	4 3231	20	0 2162
Between malf			1 2265	1	1 2265

$F_1=0\ 9265\ \ 0\ 1539=6\ 02$ at 3 and 18 d f $0\ 01>P>0\ 001$
$F_2=0\ 7766\ \ 0\ 1539=5\ 05$ at 2 and 18 d f $0\ 05>P>0\ 02$
$F_3=1\ 2265\ \ 0\ 1539=7\ 97$ at 1 and 18 d f $0\ 02>P>0\ 01$

values for the squared sinus function with and without correction for the monthly LMP distribution of normal births

Anencephaly gives no significant χ for heterogeneity and the χ^2 values do not quite reach statistical significance when Edwards formula is applied The squared sinus function however is now significant at uncorrected data and strongly significant when corrections for the normal LMP distribution are performed

Fig 1B shows the LMP distribution for this malformation

Spina bifida aperta shows a probable significance only for the squared sinus function and uncorrected data (Fig 1C) Finally *hydrocephalus* shows no significance with either χ^2 for heterogeneity Edwards model or squared sinus function

DISCUSSION

Seasonal variations of specific malformation rates have been much debated and different studies have recorded variable findings This is illustrated by Table 1 which summarizes the literature on anencephaly This table also shows that the mode of analysis has varied considerably In some studies for instance a correction was made for monthly variations in total birth rates in others not In some studies considerations have been given to differences in pregnancy length between malformed and normal infants especially in studies of anencephaly, in others not We have therefore studied the distributions recorded both concerning month of birth and month of LMP we have also studied them with and without corrections for monthly changes in birth rates We think that it cannot *a priori* be stated which mode of analysis is best as they can mirror different causes of seasonality

If factors acting in connexion with conception or at a certain relatively constant time thereafter—which should be the condition when seasonality is due to an exogenous teratogen acting at a specific stage of organogenesis—obviously the LMP month is the adequate variate to study If such a seasonality exists it will be apparent also at the time of birth provided that the gestational length of infants with the malformation involved does not deviate markedly from that of normal infants Should there be a marked deviation a distribution apparent when referred to LMP can be hidden at term if the studied material is limited An example of that was found in anencephaly at term no real seasonality could be discerned but it appeared when the study was performed on LMP dates instead If the malformed infants have normal or near normal

gestational length nothing is gained by referring the distribution to LMP Factors acting near term can be imagined as a cause of seasonality in which case the distribution at term would be less blurred than the LMP distribution A truly teratogenic factor acting at this time is in most cases improbable but factors causing for instance brain damage could be relevant Another and perhaps more important factor which would be tied to the month of birth is one that would affect the efficiency in registration of the individuals One such factor could be holiday times with less experienced or more conscientious personnel observing and reporting the infants another would be a factor that would cause a shift of pregnancy length thus for instance placing anencephalic premature infants into the category of late abortions and thereby changing the reported incidences resulting in an apparent seasonality

Corrections for monthly distribution of normal births give a more correct picture when the seasonality of a factor is studied which can hit any pregnancy with approximately the same probability This for instance is reasonably the case with a purely genetically determined malformation with full penetrance The causal factor in this case shows no seasonality but the rhythm of normal births would cause a seasonality were no correction made for it On the other hand the correction for the monthly normal birth variations can cause a false seasonality if applied on a malformation that can only—or mainly—appear in infants to a certain category of women who are excluded from the normal birth rhythms This could for instance apply to women of a certain age Such considerations must be taken into account when biological conclusions are drawn from the recorded data

Anencephaly

As Figs 1A and 1B show a seasonality does exist for anencephaly in Sweden and it is most obvious and reaches the highest statistical significance when analysed with respect to LMP distribution and with corrections for montly fluctuations in normal births

It is reasonably explained by a teratogenic *factor of one or other sort* acting during early pregnancy As anencephalic infants show a shorter and more variable gestational length than do normal infants the clearcut seasonal pattern observed for LMP will be blurred when studied at term

The seasonal rhythm of anencephalic births resembles that seen for stillbirths (7) Anencephaly contributes to only a small fraction of the total number of stillbirths and the seasonality of anencephaly cannot cause the seasonality of stillbirths in general although both phenomena could be the expression of one and the same basic phenomenon

Spina bifida aperta

The seasonal rhythm of spina bifida aperta is less conspicuous than that of anencephaly (cf Figs 1B and 1C) but a probably significant peak exists with LMP in July This is four months later than that found for anencephaly To ensure that the two recorded patterns are not random variations of one and the same pattern a variance analysis was performed as detailed in Table 6 The variance ratio F_1 in this Table shows that the two malformation groups differ significantly When this is broken up into effect of month of origin of sinus function and effect of coefficients α and β both *factors are shown to be* significant Spina bifida aperta thus has a seasonality different from anencephaly The actual graph for corrected values for spina bifida aperta (Fig 1C) suggests that two peaks exist a spring peak resembling that seen with anencephaly and an autumn peak In the uncorrected data a July peak dominates

General comments

Two malformations of the central nervous system show a seasonal pattern with an identifiable rhythm anencephaly and spina bifida aperta Peak incidences for LMP were found

Table 6 *Variance analysis comparing the squared sinus functions estimating LMP incidence of anencephaly and spina bifida aperta after correction for monthly birth rate fluctuations*
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values for the squared sinus function with and without correction for the monthly LMP distribution of normal births

Anencephaly gives no significant χ^2 for heterogeneity and the χ^2 values do not quite reach statistical significance when Edwards formula is applied *The squared sinus function however is now significant at uncorrected data and strongly significant when corrections for the normal LMP distribution are performed*

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CHRONIC ACTIVE HEPATITIS IN CHILDREN

A Clinical and Immunological Long term Study

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ABSTRACT Lidman K Biberfeld G Sterner G and Norberg R (Department of Infectious Diseases Danderyd Hospital Danderyd Department of Immunology National Bacteriological Laboratory Stockholm and the Stockholm County Council Central Microbiological Laboratory Stockholm Sweden) Chronic active hepatitis in children a clinical and immunological long term study Acta Paediatr Scand 66 73 1977.—Six girls and one boy with chronic active hepatitis (CAH) of unknown etiology were between 9 and 15 years at the clinical onset of their illness After beginning immunosuppressive therapy the course of their disease was followed from one to ten years All had markedly increased IgG high titres of smooth muscle antibodies (SMA) and antinuclear antibodies of IgG class in the earliest serum specimens tested Therapy resulted in an improved sense of well-being and a decrease in SGOT IgG and titres of SMA Very high titres of measles antibodies were observed in all cases In one of the cases CAH manifested itself after measles and in another after rubella infection The first case in our series of patients died of liver failure after 5 years of illness The other patients have survived and are able to live a normal life The possibility of CAH should be considered when children develop symptoms of hepatitis Long term immunosuppressive treatment with regular clinical and laboratory evaluation is important Estimation of titres of SMA is an additional parameter of value in *following of the* activity of CAH in these young patients

KEY WORDS Chronic active hepatitis follow up smooth muscle antibodies measles antibodies

Chronic active hepatitis (CAH) is a clinical entity characterized by a chronic progressive liver disease with superimposed episodes of activity (37) The characteristic histological appearance is that of chronic aggressive hepatitis (6) The etiology is only partly recognized It can be associated with hepatitis B infection (24) or with the use of drugs e g laxatives containing oxyphenisatin (29) But there is also a group of patients usually females with marked changes in immunological reactivity where the cause is unknown These patients often have antinuclear antibodies (ANA) (2) smooth muscle antibodies (SMA) (15) and high titres of antibodies to measles and rubella (4 16 35) All age groups may be affected by CAH but in approximately fifty per cent of the cases of unknown etiology the onset occurs between the ages of ten and thirty years (22) The response to treatment with immunosuppressive drugs is usually favourable and survival is prolonged (5 23 31)

The aim of the present study was to follow the clinical course liver function tests immunoglobulins autoantibodies and antibodies to measles and rubella in young patients with CAH on immunosuppressive therapy

MATERIAL AND METHODS

The patients studied 6 girls and 1 boy were between 9 and 15 years with an average age of 12 at the onset of illness The *mean observation* time was 5 5 years with a range of 1 5 to 10 years *All patients* were hospitalized several times and also monitored as *outpatients* every

during spring months (Febr –April) at anencephaly Spina bifida showed the most obvious peak when data not corrected for birth rate fluctuations were used A July peak was then seen For corrected data, a spring peak (April) and an autumn peak (September) are indicated The differences in peak months suggest that the described rhythms are not artefactual but are due to actual variations in malformation risk during different parts of the year Whether this is due to endogenous factors relating to fertility for instance or whether it is due to a seasonality in teratogenic factors cannot be decided

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Submitted April 20 1976
Accepted Aug 14 1976

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Treatment
1963–67 short periods with steroids *a* 1967 aza 1968 triamcinolon
1966–67 pred + aza 1967 70 aza 1970– pred + aza
1968 pred + aza
1973 no therapy
1969 pred + aza 1970– triamcinolon + aza
1970– 5 pred + aza (June) 1975– no therapy
1977 75 pred + aza (Feb)
1975– pred
1974– pred + aza

before she developed jaundice Case 7 had rubella six months before the onset of jaundice and since that infection she had persistent malaise and fatigue

There was a considerable increase in SGOT and serum IgG in the earliest specimens tested (Table 1) Serum IgA and IgM showed no apparent elevation and α_1 antitrypsin was normal HB Ag and anti HB, could not be demonstrated All patients had high titres of ANA and SMA (Table 1) The SMA were of IgG class Needle biopsies of the liver performed in case 1–6 showed lesions consistent with chronic aggressive hepatitis Biopsy had to be postponed in case 7 because of her tendency to bleed easily She also had signs of hemolytic anemia with Coombs direct and indirect tests positive Multisystem involvement was not seen in the other cases

When the diagnosis of CAH was established therapy was introduced In case 1 (Table 1) steroids were given for only short periods in the first three years This improved her sense of well being but did not control the progressive liver damage In 1967 azathioprine was added to her therapy with minimal effect The drug had to be withdrawn when the patient developed leucopenia and a Staphylococcus aureus septicaemia The infection was successfully treated but the liver function deteriorated rapidly and she bled from oesophageal varices Despite a splenorenal shunt bleeding recurred and the patient died in 1968 At necropsy the liver was moderately decreased in size with signs of severe postnecrotic cirrhosis and intense infiltration with monocytes and lymphocytes The spleen removed at the shunt operation was hypertrophic (860 g) with an abundance of plasma cells in the red pulp

Case 2–7 have received continuous immunosuppressive treatment for long periods (Table 1) This therapy has resulted in good symptomatic relief and the patients have been able to attend school or to work most of the time There was a good correlation between the decrease in levels of transaminases and the decrease in IgG and titres of ANA and SMA although the decrease in transaminases was more rapid (Table 1) Case 2 has been treated for 10 years Her disease has shown activity as measured by SGOT IgG and SMA on several occasions The galactose tolerance and bromsulphalein retention test were normal in 1972 However in 1975 the liver function was impaired and the disease showed increasing signs of activity despite therapy In case 3 therapy was discontinued after 5 years and she has remained in remission for 2 years In cases 4–7 the disease has been controlled and relapses have not occurred Bromsulphalein retention and galactose tolerance tests are normal in cases 3 4 6 and 7 and slightly abnormal in case 5 Successful liver biopsies have been performed during therapy in cases 3 and 6 These showed regression of the pathological changes to an almost normal histo

Table 1 *Representative data during the course of CAH*

The antibody titre is expressed as the reciprocal of the serum dilution n d =not determined pred =prednisolone aza= azathioprine

Case no	Age at onset	Sex	Years of follow up from onset	Time of serum sample	Bilirubin mg/100 ml	SGOT (units)	IgG (g/l)	ANA (titre)	SMA (titre)
1	15	F	5	1963	6 9	1 040	n d	n d	n d
				1967	2 7	174	42 6	800	800
				1968	4 4	40	31 8	100	200
2	9	F	10	1966	8 0	1 060	n d	n d	n d
				1967	0 4	75	41 5	400	400
				1973	0 8	18	18 4	10	10
				1975	0 8	133	33 6	100	100
3	12	F	7	1968	6 0	1 140	27 0	25	100
				1971	1 4	13	9 5	<10	<10
				1975	0 9	22	6 2	<10	<10
4	15	M	7	1968	5 4	1 120	33 2	100	100
				1972	1 0	47	17 9	25	25
				1975	1 0	40	9 6	<10	10
5	13	F	5	1970	2 7	1 080	50 8	800	400
				1971	1 1	65	15 1	50	10
				1975	1 2	40	9 1	10	<10
6	14	F	4	1972	0 8*b*	355	28 5	100	100
			4	1973	0 5	16	16 8	<10	<10
				1975	0 6	31	13 3	<10	<10
7	10	F	1 5	1974 (Sept)	4 2	982	51 4	400	400
				1975 (Jan)	0 9	82	21 4	100	25
				1975 (Dec)	0 6	39	12 9	<10	25

The disease in case 1 diagnosed 1963 Treated by us from 1967
[b] Case 6 developed jaundice in 1971 after measles infection This disappeared spontaneously before admittance to hospital

second or third month at the Department of Infectious diseases Danderyd hospital Blood samples taken on these occasions were centrifuged and stored at −20 C

Bilirubin (normal value 0 2–1 2 mg/100 ml or 4–21 μmol/l) and serum glutamic oxaloacetic transaminase (SGOT) (normal value 10–35 Karmen μ/ml or 0 08–0 28 μmols s⁻¹/l) were *regularly estimated Galactose* toler ance test (34) and bromsulphalein retention test (30) were performed The amount of α_1 antitrypsin was measured by agarose electrophoresis (14) The concentrations of immunoglobulins were estimated by single radial immuno-diffusion (20) (normal values IgG 11 ?±2 g/l IgA 1 9± 0 7 g/l IgM 0 7±0 2 g/l) Serum specimens were tested in a radioimmunoassay (AusRIA II and AusAb Abbot Laboratories Chicago Illinois *USA) for the presence of* hepatitis B surface antigen (HB_sAg) and hepatitis B sur face antibody (anti HB_s) Autoantibodies (ANA SMA) were demonstrated by indirect immunofluorescence (IFL) (17) Titres of rubella HI (32) and measles HI (25) and HLI antibodies (25) were also determined Liver biopsies were obtained by the Menghini technique (21) The histological changes were classified according to Alpert et al (1) and de Groote et al (6)

The clinical diagnosis of CAH was based on the finding of lesions consistent with chronic aggressive hepatitis on examination of the liver biopsies together with the pres ence of increased serum bilirubin prolonged elevation of transaminases considerably elevated IgG values and titres of ANA and SMA

All patients received immunosuppressive treatment Drugs used in the individual patients are shown in Table 1 In cases 2–7 the initial dose of Prednisolone given during the first week was 0 75–1 mg/kg/day This dose was then gradually reduced during a few weeks time to a mainte nance dose of 0 15–0 25 mg/kg/day (or in case 4 triam cinolone 0 04 mg/kg/day) Azathioprine when used was given in a dose of 1–2 mg/kg/day not exceeding 100 mg as a total daily dose

RESULTS

Before the onset of CAH the patients had been healthy except for asthma in early childhood in case 1 None had any known contact with viral hepatitis of type A or B or with drugs that could cause liver damage In case 1–5 the on set of disease was acute *with nausea fatigue* and jaundice Case 6 had measles one month

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Table 2 *Measles and rubella antibody titres and IgG values of serum samples during the course of CAH*

Case no	Time of clinical onset of CAH	Morbilli (clinical diagnosis)	Rubella (clinical diagnosis)	Time of serum sample	Morbilli HI (titre)	Morbilli HLI (titre)	Rubella HI (titre)	Serum IgG g/l
1	*1963*	*1962*	–	*1968*	*5 120*	*10 240*	*8*	*31 8*
2	1966	1965	Not established	1967	10 240	40 960	8	41 8
				1969	5 120	20 480	128	17 4
				1975	5 120	40 960	512	31 4
3	1968	1962	–	1968	1 280	2 560	8	17 9
				1969	320	640	8	11 5
				1975	320	640	8	7 0
4	1968	1958	–	1970	640	5 120	8	17 9
				1972	320	5 120	8	12 3
				1974	320	5 120	8	12 0
5	1970	1961	1964	1970	80	10 240	128	50 8
				1972	80	10 240	256	21 3
				1974	80	5 120	128	10 3
6	1971 (June)	1971 (May)	Not established	1971 (Dec)	5 120	10 240	256	27 5
				1972 (March)	2 560	5 120	128	22 4
				1972 (July)	640	1 280	64	12 8
				1974	320	640	32	12 3
7	1974 (August)	1967	1974 (Feb)	1974 (Sept)	12 800	12 800	12 800	51 4
				1975 (June)	3 200	12 800	6 400	21 4
				1975 (Oct)	1 600	12 800	3 200	16 5

logical pattern In cases 5 and 7 biopsy material was inadequate The hemolytic anemia was controlled in case 7 and her tendency to bleed has disappeared All patients showed very high titres of measles antibodies (Table 2) Case 7 in which the disease manifested itself after rubella infection also had high titres of rubella antibodies

DISCUSSION

The onset of CAH in children is often clinically difficult to distinguish from acute hepatitis (8) Five of seven patients had an acute onset of disease Six patients had SGOT values of approximately 1 000 when first examined which is a common finding in acute hepatitis However, the disease in the patients studied was diagnosed as CAH because of the prolonged course marked elevation of IgG values occurrence of high titres of ANA and SMA of IgG class and lesions consistent with chronic aggressive hepatitis in the liver biopsies carried out in six of the seven patients

Multisystem involvement is common in adults with CAH (12) but this was not seen among these young patients except for the occurrence of hemolytic anemia in case 7 This condition has been described previously as an unusual concomitant to CAH (26)

The IgG values were markedly elevated at the onset of the disease and decreased during immunosuppressive therapy One explanation for the hypergammaglobulinaemia in CAH and other chronic liver diseases is failure of the Kupffer cells in the diseased liver to trap antigens particularly microbial antigens from the gut resulting in increased antigen stimulation and increased antibody production (for review

see (3)) In CAH the increased antibody production may also be due to a genetically determined defect in immunoregulation It has been shown that increased titres of antibodies to measles rubella smooth muscle and nuclear antigens in patients with CAH are associated with the presence of histocompability antigens B 8 and B 12 (11) It is of interest that two cases in the present study had a viral infection shortly before onset of liver symptoms (case 6 measles and case 7 rubella) Two of the other cases had had measles the year before the onset of disease We have also observed another child (not treated at Danderyds hospital) who developed symptoms of CAH one month after measles infection (unpublished) This could suggest that these viral infections also might be of pathogenetic importance

Sera with SMA titres greater than 80 are usually derived from patients with CAH (7 13 18) In the present cases of CAH the titres of SMA were above this level at the clinical onset of the disease SMA occurring in CAH are directed against the contractile protein actin (10 18) which is present in the cytoplasm of both muscular and nonmuscular cells It is considered that during the disease process actin in the liver cells in some unknown way is made immunogenic and the production of antiactin antibodies is stimulated These antibodies do not react with the surface of normal living cells (9) and there is at present no evidence that they are cytotoxic to liver cells (28) Therefore the importance of these autoantibodies is considered primarily to be diagnostic (7)

Murray Lyon et al (23) in their controlled trial of treatment in adult patients with CAH found no apparent relationship between changes in titres of auto-antibodies including SMA and alterations in liver function tests Whittingham et al (36) however reported that SMA disappeared in 4 cases after a long remission Our experience in the present young patients is that the titre of SMA correlated well with the activity of the disease and that SMA can disappear after a long remission of the disease Therefore SMA can be an additional parameter of value in the follow up of patients

Three controlled studies mainly of adults with CAH observed for 2–5 years showed that immunosuppressive therapy reduced the activity of the disease and increased the survival (5 23 31) Dubois et al (8) in an uncontrolled study of 28 children with CAH (average age at onset 9 years and 8 months) found that the initial response to corticosteroid therapy was excellent in all but 3 patients In order to reduce side effect of corticosteroids he recommended as maintenance therapy azathioprine (1 mg/kg/day) in combination with a small dose of prednisolone

No definite conclusions about therapy can be drawn from our small series of patients However some comments can be made In case 1 the therapy did not prevent the progress of the disease This case also illustrates the risk that azathioprine may cause leucopenia White blood count must be regularly monitored when this drug is given In cases 2–7 the therapy has permitted the patients to live a normal life Case 2 has survived for ten years but her disease is now active despite therapy The other five cases are in remission three of them with therapy and two after withdrawal of therapy

The best therapeutic regime known at present is a combination of azathioprine (1–1 5 mg/kg) and prednisolone in the smallest dose which still controls the disease process The main advantage of the combination therapy is the possibility to reduce the maintenance dose and thereby the side effects of prednisolone (33) It is not known when treatment should be stopped Mackay (19) however states that it is reasonable to try to discontinue therapy when the activity of the disease remains controlled after two to three years of treatment Then there is a sustained remission in about 25% of the CAH patients 50% will require new courses of therapy and in 25% the disease seems to progress despite therapy

Table 2 *Measles and rubella antibody titres and IgG values of serum samples during the course of CAH*

Case no	Time of clinical on set of CAH	Morbilli (clinical diagnosis)	Rubella (clinical diagnosis)	Time of serum sample	Morbilli HI (titre)	Morbilli HLI (titre)	Rubella HI (titre)	Serum IgG g/l
1	1963	1962	–	1968	5 120	10 240	8	31 8
2	1966	1965	Not established	1967	10 240	40 960	8	41 8
				1969	5 120	20 480	128	17 4
				1975	5 120	40 960	512	31 4
3	1968	1962	–	1968	1 280	2 560	8	17 9
				1969	320	640	8	11 5
				1975	320	640	8	7 0
4	1968	1958	–	1970	640	5 120	8	17 9
				1972	320	5 120	8	12 3
				1974	320	5 120	8	12 0
5	1970	1961	1964	1970	80	10 240	128	50 8
				1972	80	10 240	256	21 3
				1974	80	5 120	128	10 3
6	1971 (June)	1971 (May)	Not established	1971 (Dec)	5 120	10 240	256	27 5
				1972 (March)	2 560	5 120	128	22 4
				1972 (July)	640	1 280	64	12 8
				1974	320	640	32	12 3
7	1974 (August)	1967	1974 (Feb)	1974 (Sept)	12 800	12 800	12 800	51 4
				1975 (June)	3 200	12 800	6 400	21 4
				1975 (Oct)	1 600	12 800	3 200	16 5

logical pattern In cases 5 and 7 biopsy material was inadequate The hemolytic anemia was controlled in case 7 and her tendency to bleed has disappeared All patients showed very high titres of measles antibodies (Table 2) Case 7 in which the disease manifested itself after rubella infection also had high titres of rubella antibodies

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Submitted Febr ?8 1976
Accepted July 25 1976

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ACKNOWLEDGEMENTS

We thank Drs C Lundmark previous head of the Department of Pathology Danderyd hospital for the examination of liver biopsies J Killander and A Carlstrom Department of Clinical Chemistry Danderyd hospital for the biochemical assays E Norrby Department of Virology Karolinska Institutet School of Medicine for the measles serology L Magnius Department of Virology National Bacteriological Laboratory for the hepatitis B serology and M Forsgren Stockholm County Council Central Microbiological Laboratory for the rubella serology The skilful technical assistance of Mrs Karin Knutsson Margareta Andersson and Katarina Karlen is gratefully acknowledged Financial support was received from the Swedish Cancer Society project no 799 B75 02X K Lidman received a grant from Anders Otto Swards foundation

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Acta Pædiatr Scand 66 81–84 1977

THE EFFECT OF L DOPA WITH AND WITHOUT DECARBOXYLASE INHIBITOR ON GROWTH HORMONE SECRETION IN CHILDREN WITH SHORT STATURE

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ABSTRACT Fevang F Ø Støa K F Thorsen T and Aarskog D (Department of Paediatrics and the Hormone Laboratory University of Bergen Bergen Norway) The effect of L-dopa with and without decarboxylase inhibitor on growth hormone secretion in children with short stature Acta Paediatr Scand 66 81 1977.—The efficiency of L-dopa alone and L-dopa plus a dopa-decarboxylase inhibitor (carbidopa) in provoking growth hormone (GH) secretion was studied in 40 children with short stature By preventing the extracerebral metabolism carbidopa increases the availability of L-dopa to the brain The study was designed as paired series of growth hormone stimulation tests in which the effect of L-dopa alone in different dosage schedules was compared with the same dose level of L-dopa plus carbidopa When L-dopa was given in full dose (125–500 mg) there was no significant difference in the serum GH concentrations at any time of sampling In the lower dose level the stimulant effect of L-dopa alone tended to be exceeded by the combination of L-dopa and carbidopa The serum GH responses to the different schedules indicate that an optimal hypothalamic dopamine concentration for GH release could be achieved with a considerably lower dose of L-dopa than those employed in previously reported studies When L-dopa is combined with a dopa decarboxylase inhibitor the children have the advantage of less side effects in the form of nausea and vomiting

KEY WORDS L-dopa dopa-decarboxylase inhibitor growth hormone

Dopaminergic mechanisms play a major role in hypothalamic function and are important regulators of growth hormone (GH) secretion Several studies have shown that oral L-dopa causes a significant rise in serum GH concentration and the drug has been found useful as a provocative agent in the GH stimulation test However in all studies there has been a certain number of non responders varying from 10 to 20 % of non hypopituitary subjects (6)

L dopa crosses the blood brain barrier and is converted to dopamine which functions as a neurotransmitter and mediates the secretion of GH releasing factor through activation of dopaminergic receptors The enzyme dopa decarboxylase catalyses the conversion of L dopa to dopamine both inside and outside the brain Some 95 % of an oral dose of L-dopa is decarboxylated outside the brain parenchyme in the gut wall liver kidney and other organs so that only a very small fraction actually reaches the dopaminergic receptors in the hypothalamus (2 4) The dopa decarboxylase inhibitor carbidopa inhibits peripheral utilization of L dopa and should increase the amount of L dopa available to the brain and thus possibly augment the GH response The purpose of this study was to test this hypothesis by comparing the efficiency of L dopa alone and L-dopa plus carbidopa in provoking GH secretion in children with short stature

SUBJECTS AND METHODS

The material consists of 40 children admitted to the Children s Hospital in Bergen for evaluation of short stature The purpose and nature of the study were care

time of sampling In this series there was one non responder to the reduced dose of L-dopa with carbidopa whereas all children responded to the full dose of L dopa

In the third series of 9 children who received a reduced dose of both agents there was one non responder to each test Although not significantly different both the mean GH concentration at 60 min and the mean peak concentration were higher following L dopa plus carbidopa than after L dopa alone 10 1 ng/ml versus 6 4 ng/ml ($p>0$ 40) and 14 7 ng/ml versus 9 8 ng/ml ($p>0$ 30) respectively

In Fig 1 the data from all 3 test series have been compiled according to type of drug and dose schedule L dopa alone was tested in full dose in 31 children and in reduced dose in 9 whereas L-dopa with carbidopa in reduced dose was tested in 17 children altogether The only prominent difference between the curves was a somewhat lower mean GH concentration at 60 min following the reduced dose of L-dopa 6 4±1 5 ng/ml compared to 9 6±1 3 ng/ml following full dose of L-dopa and 10 5±2 2 ng/ml following reduced dose of L-dopa plus carbidopa However the differences between these means were not significant (0 10 $<p<0$ 20) The mean peak GH concentration was 9 8±1 7 ng/ml after reduced dose of L dopa alone which was not significantly different from the mean peak response of 12 5±1 3 ng/ml (0 30$<p<0$ 20) following full dose or to the peak response of 14 6±2 1 ng/ml ($p=0$ 10) to the reduced dose of L dopa plus carbidopa

Among the 31 children who received a full dose of L-dopa there was a total of 3 non responders (10%) and an additional 3 showed a subnormal response with a peak GH concentration between 5 and 7 ng/ml Two of the 17 children receiving reduced dose of L-dopa plus carbidopa failed to respond (11 5%) and 3 showed a subnormal response

Some of the children experienced nausea and occasionally emesis during the test This unpleasant side effect occurred both when L dopa was given alone and in combination with

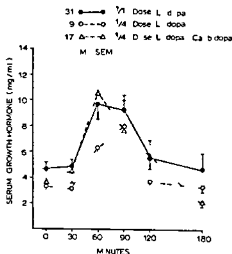


Fig 1 Serum growth hormone response to L-dopa at different dose levels and to L dopa combined with carbidopa

carbidopa However both nausea and vomiting were less frequent when the children were tested with the reduced dose of L dopa with carbidopa

DISCUSSION

A number of methods have been designed to evaluate pituitary deficiency as assessed by release of GH in response to different stimuli (6) In 1970 Boyd and co-workers reported that L dopa stimulated GH release in patients with Parkinson s disease (3) and subsequently several studies have proved the efficacy of L dopa as a reliable provocative agent for GH release in children (5 9–12) In these studies 80 to 90% of children who are not deficient in GH have responded to this stimulant These observations are confirmed and extended by our results In their original study of adult patients with Parkinson s disease Boyd and co-workers used a test dose of 500 mg of L dopa In studies involving children this test dose has either been adopted unchanged (11) or modified according to body surface area (12) or body weight (5 9 12) The basis of the different dose schedules used in the present study which was a mg per lb to mg

Table 1 *Comparison of growth hormone response (ng/ml) to L dopa and L dopa plus carbidopa* Means±S E M

Drug	Dose	No of children	Minutes 0	30	60	90	120	180	Peak
L dopa	1/1		4 8±0 4	5 1±0 6	9 9±1 6	9 1±1 4	6 4±1 2	5 2±1 6	12 5±1 6
L dopa+ carbidopa	1/1	23	3 4±0 5	7 2±1 9	9 5±1 8	11 7±3 2	7 8±1 3	4 6±0 8	15 5±3 4
L dopa	1/1		4 6±1 7	4 2±1 4	9 1±2 0	9 6±2 6	3 4±0 6	3 3±0 7	12 5±1 4
L dopa+ carbidopa	1/4	8	5 3±2 1	4 8±1 8	11 0±2 5	7 3±2 0	6 0±2 5	2 2±0 5	14 6±2 6
L dopa	1/4		3 4±1 1	3 2±0 6	6 4±1 5	7 9±2 1	3 7±0 6	3 5±0 9	9 8±1 7
L dopa+ carbidopa	1/4	9	2 3±0 4	4 3±1 0	10 1±3 5	8 1±2 4	5 3±1 4	2 1±0 2	14 7±3 [illegible]

1/1 dose 125 mg L dopa to children <15 kg 250 mg to children 15–35 kg 500 mg to children >35 kg

fully explained to the parents and their consent was obtained There were 8 girls and 32 boys whose ages ranged from 3 to 15 years With one exception all had heights below the 2 5 percentile On the basis of history clinical examination bone age X rays and endocrine studies all were considered to have non endocrine causes for their short stature

The study was designed as paired series of growth hormone stimulation tests in which the effect of L dopa alone (Larodopa® Roche) on GH release was compared with that of a combination of L dopa and the dopadecarboxylase inhibitor carbidopa (Sinemet® MSD 25 mg carbidopa per 250 mg L dopa) After an overnight fast of 8 to 12 hours an indwelling cannula was inserted into an antecubital vein and kept open with heparinized saline Following 30-min rest a single dose of the test drug was administered by mouth and blood samples were subsequently obtained after zero 30 60 90 120 and 180 min respectively for the determination of serum GH The test dose of L-dopa was based on the weights of the children Those who weighed less than 15 kg received 125 mg those 15 to 35 kg received 250 mg those above 35 kg 500 mg The tests were separated by intervals of 24 hours and alternated so that every other child had the L dopa with carbidopa test first Twenty three of the children were tested with both drugs in full dose In a second series which included 8 children the effect of the full dose of L dopa alone was compared with one fourth dose of L dopa combined with carbidopa Finally the effect of one fourth dose of both drugs was compared in another series of 9 children

The serum GH was determined by a radioimmunosorbent method (13) The HGH preparation used as standard was obtained from Calbiochem A G Switzerland and contained 2 2 I U HGH per mg

RESULTS

The mean (±S E M) serum GH concentration at each time interval together with the mean peak concentration irrespective of time are shown in Table 1 When used in full dose the response to L dopa alone and L dopa with carbidopa were fairly similar The maximum response to L dopa alone occurred at 60 min with a mean GH concentration of 9 9 ng/ml and at 90 min with a mean of 11 7 ng/ml following L dopa with carbidopa The mean GH concentration at 60 and 90 min were significantly above the zero time value following L dopa and at 60 90 and 120 min after L dopa plus carbidopa (0 001<p<0 01) The mean peak GH concentration irrespective of time was 12 5 ng/ml after L dopa and 15 5 after L dopa with carbidopa There was no significant difference between these mean values or those at any individual time of sampling When a peak GH concentration of 5 ng/ml or more was arbitrarily considered to indicate a normal response there were two non responders to L dopa alone whereas one did not respond to the combination of L dopa and carbidopa One child who did not respond to either drug subsequently showed a normal response to an insulin provocation test

In the series of 8 children tested with the full dose of L dopa and a quarter dose of L dopa plus carbidopa the maximum response to both agents occurred at 60 min with a mean GH concentration of 9 1 ng/ml respectively 11 0 ng/ml There was no significant difference between these mean values or those at any other

time of sampling In this series there was one non responder to the reduced dose of L-dopa with carbidopa whereas all children responded to the full dose of L dopa

In the third series of 9 children who re ceived a reduced dose of both agents there was one non responder to each test Although not significantly different both the mean GH concentration at 60 min and the mean peak concentration were higher following L dopa plus carbidopa than after L dopa alone 10 1 ng/ml versus 6 4 ng/ml ($p>0$ 40) and 14 7 ng/ml versus 9 8 ng/ml ($p>0$ 30) respectively

In Fig 1 the data from all 3 test series have been compiled according to type of drug and dose schedule L dopa alone was tested in full dose in 31 children and in reduced dose in 9 whereas L dopa with carbidopa in reduced dose was tested in 17 children altogether The only prominent difference between the curves was a somewhat lower mean GH concentra tion at 60 min following the reduced dose of L-dopa 6 4±1 5 ng/ml compared to 9 6±1 3 ng/ml following full dose of L dopa and 10 5± ?? ng/ml following reduced dose of L dopa plus carbidopa However the differences be tween these means were not significant (0 10 $<p<0$ 20) The mean peak GH concentration was 9 8±1 7 ng/ml after reduced dose of L dopa alone which was not significantly dif ferent from the mean peak response of 12 5± 1 3 ng/ml (0 $30<p<0$ 20) following full dose or to the peak *response of* 14 6±2 1 ng/ml ($p=0$ 10) to the reduced dose of L dopa plus carbidopa

Among the 31 children who received a full dose of L dopa there was a total of 3 non responders (10%) and an additional 3 showed a subnormal response with a *peak GH* con centration between 5 and 7 ng/ml Two of the 17 children receiving reduced dose of L dopa plus carbidopa failed to respond (11 5%) and 3 showed a subnormal response

Some of the children experienced nausea and occasionally emesis during the test This unpleasant side effect occurred both when L dopa was given alone and in combination with

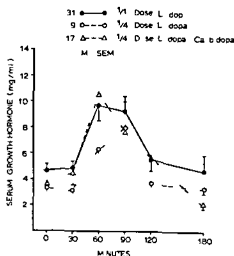


Fig 1 Serum growth hormone response to L-dopa at different dose levels and to L dopa combined with carbi dopa

carbidopa However both nausea and vomit ing were less frequent when the children were tested with the reduced dose of L dopa with carbidopa

DISCUSSION

A number of methods have been designed to evaluate pituitary deficiency as assessed by release of GH in response to different stimuli (6) In 1970 Boyd and co-workers reported that L dopa stimulated GH release in patients with Parkinson s disease (3) and subsequent ly several studies have proved the efficacy of L dopa as a reliable provocative agent for GH release in children (5 9–12) In these stud ies 80 to 90% of children who are not defi cient in GH have responded to this stimulant These observations are confirmed and ex tended by our results In their original study of adult patients with Parkinson s disease Boyd and co-workers used a test dose of 500 mg of L dopa In studies involving children *this test* dose has either been adopted un changed (11) or modified according to body surface area (12) or body weight (5 9 12) The basis of the different dose schedules used in the present study which was a mg per lb to mg

per kg conversion of the 125 to 500 mg dose proposed by Weldon and co workers (12) represents a dose in the lower range of those used in the different studies in children

Animal experiments have indicated that only a small fraction of orally administered L dopa enters the brain and that this portion is significantly increased by the concurrent use of systemic decarboxylase inhibitors (1) These experimental results are in accordance with clinical experience in patients with Parkinson s disease where the dose of L dopa required for optimum therapeutic benefit can be reduced when L dopa is combined with carbidopa (8)

When used in the highest dose level L dopa with carbidopa did not augment the GH release above that obtained with L dopa alone This finding might infer that the hypothalamic dopaminergic receptors involved in the modulation of GH releasing factors react to a threshold concentration of dopamine or to concentrations up to a certain limit and that further increase in the dopamine level will not enhance the stimulus In the lower dose level employed in the present study stimulant effect of L dopa alone tended to be exceeded by L dopa combined with carbidopa The responses to the different schedules indicate that an optimal hypothalamic dopamine concentration for GH release could be achieved with a considerably reduced dose of L dopa when combined with a dopa decarboxylase inhibitor (Fig 1)

The children who received the reduced dose of L dopa plus carbidopa had the advantage of less nausea and vomiting than those tested with full dose of L dopa alone Nausea and vomiting are probably due to the effect of dopa metabolites on the medullary emetic zone, possibly the area postrema which lies outside the blood brain barrier for dopamine (7) The reduction of these side effects could probably be due to carbidopa blocking the formation of dopamine in extracerebral tissue thus decreasing the degree of dopaminergic stimulation of the medullary emetic zone

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Submitted May 26 1976
Accepted Aug 21 1976

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Acta Pædiatr Scand 66 85–89 1977

LOW MOLECULAR WEIGHT ORGANIC ACIDS IN THE URINE OF THE NEWBORN

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ABSTRACT Gregersen N Ingerslev J and Rasmussen K (Biochemical Research Unit University Department of Clinical Chemistry and University Department of Obstetrics and Gynaecology Århus Kommunehospital Århus Denmark) Low molecular weight organic acids in the urine of the newborn Acta Paediatr Scand 66 85 1977 —The urinary excretion of seven selected low molecular weight organic acids in normal neonates was measured by gas chromatography First and third to fourth day of life excretion of the following compounds was significantly unchanged 3-OH butyric acid (<13 μmol/mmol creatinine) succinic acid (approx 43 μmol/mmol creatinine) adipic acid (approx 12 μmol/mmol creatinine) 2-OH-glutaric acid (approx 23 μmol/mmol creatinine) 3-OH 3 Me-glutaric acid (approx 25 μmol/mmol creatinine) and citric acid (approx 115 μmol/mmol creatinine) The excretion of 4-OH phenyl-acetic acid increased during the first four days of life (from <8 μmol/mmol creatinine to approx 20 μmol/mmol creatinine) It is postulated that urinary organic acid excretion in the neonate which is clearly different from the adult urinary pattern is a reflection of the specific neonatal metabolic situation including a high fatty acid utilisation and a low protein catabolism

KEY WORDS Low molecular weight organic acids succinic acid adipic acid citric acid neonatal metabolism

Information concerning the normal urinary excretion of low molecular weight organic acids during the first days of human life was until recently very limited Karoum et al (8) investigated the content of phenolic acids in the urine of normal neonates and very recently Bjorkman et al (2) analysed the urinary pattern of organic acids in first day neonates The purpose of the present investigation was to obtain reference values for the concentrations of a selected number of urinary metabolites exceeding approximately 8 μmol/mmol creatinine i e the practical detection limit for precise measurement by the method employed

MATERIAL AND METHODS

Clinical material

Urine was collected from 18 full term neonates (10 males and 8 females) in two periods immediately post partum and on the third to fourth day of life In an additional 10 cases (3 males and 7 females) only a first day portion was obtained

All mothers were healthy on physical examination prior to labour and in no instance signs of preeclampsia were recorded Systemic medications were not given for at least 4 weeks before term In 4 cases a single dose of Menadion (40 mg) was given at onset of labour Two mothers were given pudendal anaesthesia and all received N_2O No other medical treatment was instituted during labour

Duration of labour varied from 1 h 10 min to 8 h 45 min In 26 cases the delivery was by cephalic presentation and in 2 instances by breech

Amniotic fluid and all placentas were normal All infants weighed more than 2 500 g at birth The Apgar score was 10 in all cases 1 min after birth No signs of late asphyxia were recorded and jaundice was not seen Most infants were given minor quantities (?0–40 ml per day) of either glucose (5%) or diluted cow s milk (50%) until lactation was firmly established Weight losses for the first 5 days ranged from 450 g to zero (mean 170 g)

Chemicals

DL 3-OH-butyric acid (Na-salt) succinic acid and 4-OH phenylacetic acid were obtained from Merck A G (Darm

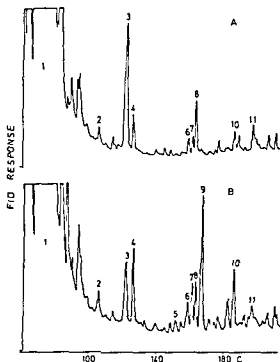


Fig 1 Typical GC profile from neonate girl A first day B third day 1 solvent front 2 3-OH butyric acid 3 urea 4 succinic acid 5 adipic acid 6 2-OH glutaric acid 7 3-OH 3 Me-glutaric acid 8 pimelic acid (int st) 9 4 OH phenylacetic acid 10 citric acid 11 hippuric acid

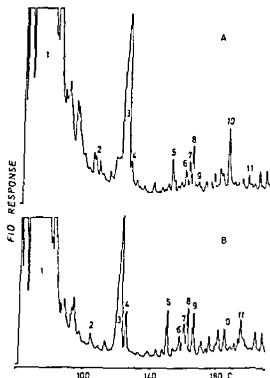


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Analytical procedure

Urine samples containing 2 mg (17 5 μmol) creatinine were diluted with equal amounts of saturated NaCl solution and 200 μl of a 400 mg/l (2 5 mmol/l) pimelic acid solution The pH of the mixture was adjusted to 1 with 5 mol/l HCl prior to extraction Three consecutive extractions were made with both ethylether and ethylacetate The organic phases were dried (Na_2SO_4) and the solvent was evaporated in a stream of dry nitrogen The remanence was silylated to TMS ethers/esters at room temperature with Tri Sil (200 μl) and BSTFA (200 μl) 2–8 hours after silylation 4 μl of the mixture was injected into a Hewlett Packard 5830 gas chromatograph The instrument was equipped with a flameionisation detector an integrator and a dedicated computer The column was a 1 8 m×3 mm (i d) glass coil packed with Dexsil 300 (3 %) on Chromosorb W (HP) The temperature of the column was programmed at 4 C/min from 60 C The helium carrier flow rate was 40 ml/min

An aqueous stock solution (50 ml) consisting of 3-OH butyric acid (Na salt 30 mg 265 μmol) succinic acid (25 mg 212 μmol) adipic acid (25 mg 171 μmol) 2 OH glutaric acid (Zn salt 45 mg 212 μmol) 3 OH 3 Me glutaric acid (50 mg 308 μmol) 4 OH phenylacetic acid (25 mg 164 μmol) and citric acid (125 mg 647 μmol) was used as standards in appropriate dilutions

The standards were analyzed according to the procedure described for urines Standard curves of the compounds were straight and through zero The variation coefficients calculated from double determinations on several concentration levels in the standards were 3 OH butyric acid 12% succinic acid 15% adipic acid 9% 2 OH glutaric acid 7% 3 OH 3 Me glutaric acid 7% 4 OH phenylacetic acid 4% and citric acid 8% It was only possible in a few cases to make double determinations on the urine specimens from the children However these analyses indicated that the variation coefficients were in the same order of magnitude as in the standards from 10–15%

The chemical structure as well as the gas chromatographic retention time of the internal standard pimelic acid are so similar to those of the compounds in question that the variation component due to variations in the extraction efficiency are minimal Recovery of the compounds was not quantitative but extraction efficiency was normalised with reference to pimelic acid

Table 1 *Low molecular weight organic acids excretion in humans*

	Neonate urinary excretion of organic acids							
	Present study				Bjorkman et al (2) day 1		Adult excretion of organic acids Bjorkman et al (2)	
	♂+♀ day 1		♂+♀ day 3–4					
	Range	Median	Range	Median	Range	Mean	Range	Mean
3-OH Butyric acid								
µmol/mmol creatinine	nd –79	nd	nd–31	nd	Not reported		Not reported	
µg/mg creatinine	nd–23	nd	nd–75	nd				
Succinic acid								
µmol/mmol creatinine	13–101	45	24–83	39	14–295[a]	83	2–9[a]	5
µg/mg creatinine	13–105	47	25–86	40	14–305	86	2–9	5
Adipic acid								
µmol/mmol creatinine	nd–59	15	nd–46	12	1–27	9	2–7	4
µg/mg creatinine	nd–76	19	nd–59	15	1–34	11	2–9	5
2-OH-glutaric acid								
µmol/mmol creatinine	11–70	28	nd–36	22	Not reported		Not reported	
µg/mg creatinine	14–91	36	nd–47	28				
3-OH 3 Me glutaric acid								
µmol/mmol creatinine	nd–62	25	10–56	25	6–74	27	1–3	1
µg/mg creatinine	nd–88	36	14–80	35	9–105	38	1–4	2
4-OH *phenylacetic acid*								
µmol/mmol creatinine	nd–31	nd	nd–160	30	1–16	9	5–22	11
µg/mg creatinine	nd–41	nd	nd–213	40	2–21	12	7–30	15
Citric acid								
µmol/mmol creatinine	14–797	128	20–474	101	7–170	42	12–73	27
µg/mg creatinine	23–1 350	216	34–717	171	12–787	71	20–123	46

nd not detectable limit of detection 10 µg/mg creatinine
[a] Total of succinic acid and fumaric acid

Gas chromatography/mass spectrometry

Identification and verification of the detected compounds in urine samples were performed on a AEI MS 30 *double* beam mass spectrometer equipped with a Pye Unicam gas chromatographic inlet system

RESULTS

Typical gas chromatographic profiles of low molecular weight organic acids *from two* neonatal urines are shown in Figs 1 and 2

The profiles are rather complex A *pilot in*vestigation indicated that 3-OH butyric acid succinic acid adipic acid 2-OH glutaric acid 3-OH 3 Me glutaric acid 4 OH phenylacetic acid were identifiable and measurable in al most all pilot urines whereas pimelic acid later used as internal standard was *not de*tected The identification and quantitation of these acids were performed by gas chromatography/computer matching of the unknown profiles to a profile of known amounts of the *acids in question In order to check* the gas chromatographic procedure the identity of the acids in some urine samples was verified in the gas chromatographic/mass spectrometric *system*

The results were allocated to 4 groups 1) *boys first day of life* 2) boys day 3–4 3) girls first day 4) girls day 3–4

The detected concentrations of each acid were not found to be normally distributed within the groups (histogram technique) The nonparametric Wilcoxon tests for two samples (5) showed no significant differences between either groups 1 and 3 or between 2 and 4 The Wilcoxon test of paired differences (5) applied to the excretions on day 1 and day 3–4 matching groups 1+3 to groups 2+4 showed a significantly enhanced excretion of 4 OH phenyl acetic acid ($2\alpha < 0.01$) The median values are also clearly different (Table 1)

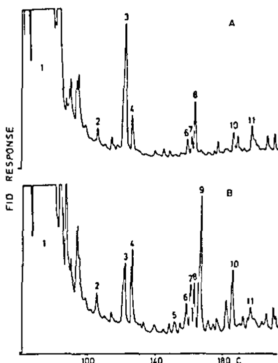


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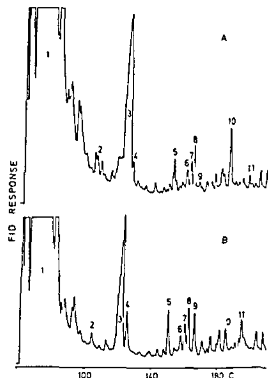


Fig 2 Typical GC profile from neonate boy A first day B third day 1 solvent front 2 3-OH butyric acid 3 urea 4 succinic acid 5 adipic acid 6 2-OH-glutaric acid 7 3 OH 3 Me glutaric acid 8 pimelic acid (int st) 9 4-OH phenylacetic acid 10 citric acid 11 hippuric acid

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Submitted March 29 1976
Accepted June 29 1976

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DISCUSSION

During the first hours of life glycogen stores are depleted (15) Thereafter, the primary source of calories is endogen fat This is reflected in the rapid rise in blood lipids (11) Protein catabolism contributes little to energy requirements (4) This particular metabolic situation seems to be reflected in the urinary excretion of non nitrogen organic acids as shown in Table 1 The excretion of the metabolites in the neonate is remarkably different *from the excretion in the adult* (2, 17) Comparing the results of Bjorkman et al (2) and the results obtained in the present study major disagreements are found as regards the excretion of succinic acid and citric acid (Table 1) In the work of Bjorkman et al succinic acid and fumaric acid did not separate in the gas chromatographic profile This fact might explain the difference in the concentrations of succinic acid No satisfactory explanation for the difference in citric acid excretion is available However, the nutritional condition of mother and child might have been different *Excretion of the organic acids in* vestigated in our study did not differ between the two days of collection with the exception of 4 OH phenylacetic acid the excretion of which increased *during the first four days* This most likely due to production by the establishing intestinal flora (6)

Taking into consideration the high blood concentration of fatty acids and ketone bodies in the newborn (11) the excretion of 3 OH *butyric acid in the first days of life* is remarkably low The reported levels of fatty acids and ketone bodies (11) are comparable to those in adult blood after one to two days of hunger, where substantial amounts of urinary 3 OH butyric acid are found (13 14) The impaired ability to develop ketonuria in the first period of life has been demonstrated by other investigators (7, 16) but the biological mechanism is still not understood

Succinic acid *is excreted in a variety of* ketotic conditions (12) and ketoacidosis in the neonate is accompanied by excretion of succinic acid (9)

The substantial amounts of adipic acid in the urines investigated most probably reflect the high lipolytic activity and increased catabolism *of fatty acids in the neonate* (11) This assumption is based on the observations of Pettersen et al (12) showing a positive correlation between the degree of ketosis and the excretion of adipic acid

In the neonate high plasma concentration (10) and urinary excretion (1, 2 3) of citric acid have been reported

The biological significance of the metabolic profiles reported awaits further investigations

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Acta Pædiatr Scand 66 91–96 1977

TEMPORARY INTESTINAL LACTASE DEFICIENCY IN LIGHT TREATED JAUNDICED INFANTS

A F BAKKEN

From the Department of Paediatrics Rikshospitalet University of Oslo Oslo Norway

ABSTRACT Bakken A F (Department of Paediatrics Rikshospitalet University of Oslo Oslo Norway) Temporary intestinal lactase deficiency in light treated jaundiced infants Acta Paediatr Scand 66 91 1977.—The intestinal lactase activity in six newborn jaundiced light treated infants with diarrhea and in eight normal controls were compared by lactose tolerance test (LTT) The ability to hydrolyze lactose was minimal in the jaundiced infants during light treatment compared to the controls which could absorb lactose very well Peroral intestinal biopsies were taken from the newborn jaundiced infants during light treatment By histochemical technique no intestinal lactase activity was found in these intestines When the jaundiced infants with diarrhea were given lactose free diet the stools normalized The effect was reversed when breast milk was given while the baby was still jaundiced and light treated These findings indicate that the increased amounts of unconjugated bilirubin in the intestine of jaundiced infants during light treatment inhibit the intestinal brush border lactase When the icterus fades the lactase is again active The practical consequence is to give light treated infants lactose free diet if they get diarrhea and to reintroduce breast milk or other lactose containing diet when the baby is no longer icteric

KEY WORDS Light treatment intestinal lactase intestinal biopsy diarrhea in the newborn

Ever since Cremer et al (6) introduced light treatment of the newborn jaundiced infants loose greenish stools from some of these have been observed (18) The current explanation for these loose stools is vague they are said to be due to increased amounts of break down products from bile pigments excreted in the bile (18 19) However serious dehydration of these infants due to the diarrhea has not been reported and light treatment of jaundiced neonates is used throughout the world in spite of some of the disadvantages which have been described (20 24)

It has been found that the concentration of unconjugated bilirubin is increased in the bile and intestine during light treatment of jaundiced infants (4 19 21) Furthermore it has been shown that unconjugated bilirubin is able to uncouple oxidative phosphorylations and thereby decrease the energy formation intracellularly (5 29) An attractive hypothesis therefore is that the cause of the diarrhea in these infants might be a temporary inhibition of the intestinal brush border lactase by the increased amounts of unconjugated bilirubin in the intestine

The present study was performed to see if this theory could be substantiated different groups of neonates had their ability to hydrolyze and absorb lactose investigated Diets both containing lactose and lactose free were given to see the effect upon the stools of the newborn babies

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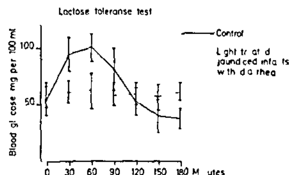


Fig 1 Lactose tolerance test (LTT) performed on 6 new born jaundiced infants with diarrhea during light treatment LTT from 8 normal controls are shown for comparison ±one standard deviation is shown for each value

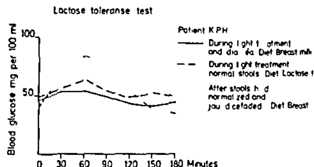


Fig 2 Lactose tolerance test performed on one newborn jaundiced infant at three different occasions during light treatment while the patient had diarrhea and was given breast milk during light treatment while the stools were normalized because of a lactose free diet and after the jaundice had faded the stools were normalized and breast milk was reintroduced

MATERIALS AND METHODS

Six newborn caucasian infants with physiological jaundice (no Rhesus or ABO incompatibility) were studied They all had serum bilirubin above 10 mg/100 ml (measured by Jendrassik & Gróf's method (13) on the second day of life and they were put under light (130 footcandles light unit equipped with 10 white 20 Watt fluorescent bulbs) During the light treatment these six infants developed diarrhea (more than six loose stools per 24 hours) Peroral intestinal biopsy was performed on these infants during the second or third day of life A double loop hydraulic capsule was used (2) and the biopsies were taken close to the ligament of Treitz The biopsies were investigated with hematoxiline/eosine staining and histochemically for brush border lactase using the method described by Lojda et al (17) Lactose tolerance tests (LTT) were performed on these six infants during the second or third day of life Because of the possible false results obtained by oral administration of lactose (16) lactose in a concentration of 2 g/kg body wieght of the infants was installed directly into the stomach Blood glucose was measured by Hultman s method (12) at times zero 30 60 90 120 150 and 180 minutes after the lactose had been given

After two days in the light while the diarrhea still persisted these six infants were given lactose free diets (AL 110® Nestlé) for three days After this period breast milk was reintroduced Their numbers of stools were observed over this period

A LTT was also performed in three of these infants during the period of lactose free diet and another after the jaundice had faded and the stools had normalized on breast milk

Eight normal newborn babies were used as controls These infants were all of the same race and in the same weightrange as the jaundiced infants (3–4 kg) however none of them had serum bilirubin above 10 mg/100 ml at any time and none had diarrhea during the first week of life They were all fed exclusively on breast milk A LTT was performed on these infants during the second or third day of life Peroral intestinal biopsies were not performed

Normal intestinal brush border lactase was looked for by the same histochemical technique of Lojda et al (17) in a two-year-old girl whose coeliac disease was now controlled after a period of gluten free diet

To test the effect of bilirubin on the intestinal lactase in vitro slices from the biopsy specimen of the normal intestine were incubated in a solution of bilirubin (15 mg/100 bilirubin Hoffman La Roche B 509289 in isotonic sodiumbicarbonate pH (8 3)) for 30 minutes prior to the application of the histochemical technique

RESULTS

Lactose tolerance test

Fig 1 shows the results of the LTT performed in the light treated jaundiced infants during their period of diarrhea on the second or third day of life The LTT from the control infants are shown for comparison

Fig 2 gives the results from the LTT performed on one of the six jaundiced light

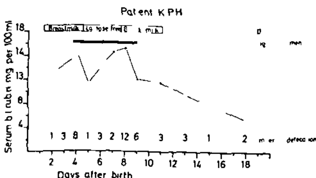


Fig 3 The numbers of defecations and the serum bilirubin level are shown in a patient with physiological jaundice The duration of light treatment and the different diets in this period are indicated



Fig 4 Duodenal mucosa from a two day old jaundiced light treated infant with diarrhea (Hem eos x 50)

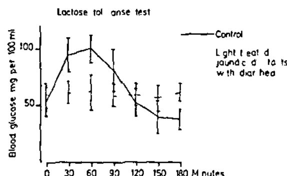


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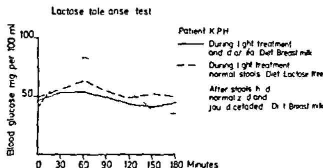


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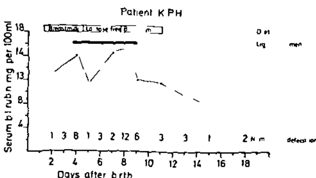


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Fig 5 Duodenal mucosa from a two year old infant The mucosa is investigated histochemically for brush border lactase The enzyme activity is shown as black colour at the intestinal villi (×36)

treated infants at three different occasions During light treatment while the diet was breast milk and the stools were loose during light treatment after the stools had normalized and the infant was on lactose free diet and after the jaundice had faded and the infant was back on breast milk The details of the observations from the same baby are shown in Fig 3 The serum bilirubin levels are indicated from day 2 to day 18 after birth the number of defaecations each day recorded and the diet on the different days and the period of light treatment are shown The drastic reduction in the number of defaecations and at the same time a normalizing of the stools when lactose free diet was given instead of breast milk was typical for all the six infants in this group The reappearance of loose stools when breast milk was reintroduced with the infant still on light treatment was also a common finding

Intestinal biopsies

A typical picture of the mucosa from the duodeno-jejunal region of a two-day old jaundiced infant is shown in colour in Fig 4 The mucosa is icteric and the villi are very different from what we are used to see in normal though older infants (see Fig 5 for comparison) The villi are almost absent or at least rudimentary The surface epithelium is cuboidal containing mucous as are the glands inside the mucosa There is no cellular infiltration This picture of a yellow mucosa and almost no normal villi was a constant finding in the jaundiced light treated infants during their diarrheal periods The application of the histochemical method for lactase is shown in Figs 5 and 6 Fig 5 shows the activity of lactase as black colour on the surface of the villi in the duodenal mucosa of a two-year old girl whose mucosa was normalized after 9 months on gluten free diet Fig 6 reveals no lactase activity in the mucosa of one of the newborn jaundiced infants with diarrhea While Fig 5 shows long normal villi in the two year old girl Fig 6 shows as in Fig 4 an almost total lack of normal villi in the jaundiced infant with icteric mucosa

When the biopsy specimen of the normal two year old girl was pretreated with bilirubin in vitro for 30 minutes no lactase activity was found afterwords while the structure of the mucosa remained intact

DISCUSSION

The difference between the absorption of lactose in jaundiced infants during diarrheal periods when on light treatment and normal infants (Fig 1) is significant It has been found



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give energy to hydrolyzing reactions deteriorates Another way of inhibition of the lactase by the unconjugated bilirubin may be through reduction of ATP ase activity (19) or an effect more basically on the nucleic acids (24)

Supporting the results presented in this paper are the findings of Yeung (28) He describes a significant inverse correlation between the serum bilirubin level and the blood sugar in the first four days of life This might be an indirect clue to the inhibition of the intestinal lactase by unconjugated bilirubin

Regardless the mechanism of lactase dysfunction during light treatment of the newborn baby the increased amount of unconjugated bilirubin in the intestine during light treatment makes lactose-containing diet unsuitable for these babies and such food ought to be replaced by lactose free diet to avoid possible complications from the loose stools The mothers should be encouraged to pump out their milk during this period and be told that the breast feeding can be started again as soon as the baby is out of the light box

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Fig 6 Duodenal mucosa investigated histochemically for brush border lactase in a two day old jaundiced light treated infant with diarrhea No activity is seen (for normal activity see Fig 5) (×36)

by other investigators (15 21) that there is a fairly good correlation between LTT and the lactase activity in the intestine measured by non histochemical means It is therefore obvious that there is an impaired ability in the jaundiced light treated infants to absorb lactose This disability is not exclusively due to increased rate of transport of the intestinal content, as Fig 2 shows the LTT is lower even if the stools are normal on lactose free diet as long as the infant is jaundiced

Furthermore the flat LTT of the jaundiced newborn is obviously abnormal when compared to normal newborn infants and even premature babies (Fig 1 (3 15)) Boellner et al (3) showed that the LTT might be influenced by the amount of lactose given in the period before the LTT is performed This cannot play an important part in a two day old infant However, the inductibility of intestinal brush border lactase is still an object of discussion (10, 16)

The great variation in the number of stools with the different types of diet is shown in Fig 3 The number of stools normalized when lactose was excluded from the diet This is an indirect clue for what is shown by the LTT In these jaundiced light treated newborn infants the ability to hydrolyze lactose is minimal

The complete lack of intestinal lactase activity shown in Fig 6 is in contrast to what has been found in spontaneous abortions and in stillborn babies (1 8) This was anticipated from studies of the structure of the intestine as it has been found that the lactase activity is localized strictly to the brush border epithelium (7) and that the lactase activity is especially vulnerable when structural changes occur in the intestinal epithelium (11) To verify this intestinal biopsies from normal newborn infants ought to have been studied However I did not feel I had the ethical right to perform such investigations on normal babies even if the complications from intestinal biopsies generally are very rare (27)

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Submitted April 12 1976
Accepted Aug 11 1976

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Acta Pædiatr Scand 66 97–102 1977

CONCENTRATION OF THYROGLOBULIN IODINE CONTENTS OF THYROGLOBULIN AND OF IODOAMINOACIDS IN HUMAN NEONATES THYROID GLANDS

NICOLE ETLING

From the Unite 30 INSERM Hopital des Enfants Malades Paris France

ABSTRACT Etling N (Unite 30 INSERM Hopital des Enfants Malades Paris France) Concentration of thyroglobulin iodine contents of thyroglobulin and iodoamino acids in human neonates thyroid glands Acta Paediatr Scand 66 97 1977.—The iodine and protein concentrations the iodoamino acids content of thyroglobulin (TG) were determined in 17 thyroid tissues from human neonates who died from 3 hours to 47 days after birth Total iodine concentration of neonate tissues increased with life duration TG concentration was related to the survival duration of the neonates Increase of the iodine content was associated to the increase of the TG content mean value was 0 16 μg ^{127}I/100 μg TG in neonates who died within the first 20 hours after birth 0 25 μg in neonates who survived 26 to 72 hours and 0 43 μg in neonates living more than 10 days Expressed as iodine content to total iodine ratio iodoamino acid percentages were not related to the iodine concentration of the tissue When the iodoamino acids were expressed in residues per molecule of TG iodotyrosines thyroxine and triiodothyronine increased with duration of survival These variations in iodine content and concentration of thyroglobulin could be related to acute hormonal changes in serum observed in early neonatal period

KEY WORDS Newborn thyroid tissue iodoamino acids thyroglobulin

In the human newborn the immediate and early post natal period is associated with a variety of acute changes in several aspects of thyroid hormone economy Within the first few minutes of extra uterine life serum thyrotrophin (TSH) concentration increases markedly (10 4) several hours later the serum 3 5 3 triiodothyronine (T_3) rises sharply (1) followed by an increase in serum thyroxine (T_4) (5) Knowledge of these alterations is derived entirely from the numerous measurements that have been made in the blood Little is known in contrast about the changes which may be occuring within the thyroid gland during the same general period In 1938 Palmer (17) using a microchemical technique measured the total iodine and T_4 content of the thyroid in a series of 7 month fetuses to 12 days old infants The ratio of T_4 iodine to total iodine was found to be essentially the same as in normal adults Recently Fisher and co-workers (11) using radioimmunoassay studied the ratio of T_4 to T_3 in the thyroid gland of abortuses the oldest of which were at 26 and 31 weeks of gestation In all the ratios were similar to those reported in euthyroid adults

We have recently shown (8) that in neonatal thyroid tissue both the colloid content and the iodine content of extracted proteins displays transient variations during the neonatal period These variations appear to be more closely related to survival time following birth than to gestational age at birth In the present report we examine the concentration of thyro

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ABSTRACT Etling N (Unite 30 INSERM Hopital des Enfants Malades Paris France) Concentration of thyroglobulin iodine contents of thyroglobulin and iodoamino acids in human neonates thyroid glands Acta Paediatr Scand 66 97 1977.—The iodine and protein concentrations the iodoamino acids content of thyroglobulin (TG) were determined in 17 thyroid tissues from human neonates who died from 3 hours to 47 days after birth Total iodine concentration of neonate tissues increased with life duration TG concentration was related to the survival duration of the neonates Increase of the iodine content was associated to the increase of the TG content mean value was 0 16 μg I/100 μg TG in neonates who died within the first 20 hours after birth 0 25 μg in neonates who survived 26 to 72 hours and 0 43 μg in neonates living more than 10 days Expressed as iodine content to total iodine ratio iodoamino acid percentages were not related to the iodine concentration of the tissue When the iodoamino acids were expressed in residues per molecule of TG iodotyrosines thyroxine and triiodothyronine increased with duration of survival These variations in iodine content and concentration of thyroglobulin could be related to acute hormonal changes in serum observed in early neonatal period

KEY WORDS Newborn thyroid tissue iodoamino acids thyroglobulin

In the human newborn the immediate and early post natal period is associated with a variety of acute changes in several aspects of thyroid hormone economy Within the first few minutes of extra uterine life serum thyrotrophin (TSH) concentration increases markedly (10 4) several hours later the serum 3 5 3 triidothyronine (T_3) rises sharply (1) followed by an increase in serum thyroxine (T_4) (5) Knowledge of these alterations is derived entirely from the numerous measurements that have been made in the blood Little is known in contrast about the changes which may be occuring within the thyroid gland during the same general period In 1938 Palmer (17) using a microchemical technique measured the total iodine and T_4 content of the thyroid in a series of 7 month fetuses to 12 days old infants The ratio of T_4 iodine to total iodine was found to be essentially the same as in normal adults Recently Fisher and co-workers (11) using radioimmunoassay studied the ratio of T_4 to T_3 in the thyroid gland of abortuses the oldest of which were at 26 and 31 weeks of gestation In all the ratios were similar to those reported in euthyroid adults

We have recently shown (8) that in neonatal thyroid tissue both the colloid content and the iodine content of extracted proteins displays transient variations during the neonatal period These variations appear to be more closely related to survival time following birth than to gestational age at birth In the present report we examine the concentration of thyro

28 Yeung C Y Blood sugar changes in neonatal hyper bilirubinemia and phenobarbitone therapy *Arch Dis Child 47* 246 1972
29 Zetterstrom R & Ernster L Bilirubin an uncoupler of oxidative phosphorylation in isolated mitochon dria *Nature 178* 1335 1956

Submitted April 12 1976
Accepted Aug 11 1976

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Table 2 *Means and standard deviations of iodine and iodoamino acid content of thyroglobulin (TG) in thyroid tissue from human neonates*

Group	TG iodine (μg ^{127}I/ 100 μg TG)	Iodoamino acid content (residues/mole TG)			
		MIT	DIT	T	T_3
I	0 16±0 11	2 35±1 41	1 71±1 27	0 61±0 44	0 03±0 03
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III	0 43±0 05	5 85±0 59	4 18±0 59	1 84±0 38	0 30±0 03

analysis because of the far lesser sensitivity of the latter

The second gel of the pair was stained with 0 5% amidoblack in 2% acetic acid and destained with 7% acetic acid until complete decoloration of the background was obtained The stained proteins were scanned in a Gilford spectrophotometer at 610 nm using a recorder fitted with a linear transport attachment The percentage of thyroglobulin was calculated either from the weight of several protein peaks found on the scan itself or by planimetry and good proportionality between the height of a peak and content of protein was obtained with quantities of protein between 5 and 40 μg

For the measurement of the absolute concentration of the several iodoamino acids the small amount of thyroid tissue available and its low iodine content precluded the use of classical techniques and an alternate method of greater sensitivity was employed Hydrolysis of the NaCl soluble extracts was performed either with Pronase (13) or with Pronase Leucylamino-peptidase (18) there was no difference between the results of the two digestion techniques probably because of the low iodine content of the extracts An aliquot of the hydrolysate containing around 100 ng iodine was applied to Whatman 1 paper together with μl bromophenol blue (1 mg/ml) to serve as a marker Chromatograms were developed in *n* butanol acetic acid water (78 5 17) and *n* butanol saturated with ? N ammonium hydroxide After chromatography each strip was cut into 1×3 cm segments and these were ashed in separate tubes using the sulfonitroperchloric mixture Iodine contents were then measured as described earlier The ng iodine values were calculated from the spectrophotometric peaks and the percentages evaluated Recovery of the known amounts of the iodine applied to each chromatographic strip was nearly complete

The percentage of iodide in the soluble extracts was also evaluated by paper electrophoresis (1 hour at 300 v in 0 M ammonium carbonate pH 8 4) ^{127}I determinations were done as previously described and good concordance with the results of chromatography was observed

All chromatographic electrophorectic and iodine analyses were performed at least twice for each specimen

RESULTS

Iodine and thyroglobulin concentrations

The total iodine concentration in the thyroids of neonates was far lower than in thyroid tissue of adults (Table 1) Mean values were lowest in Group I (6 25±4 96 μg/100 mg mean ±S D) increased slightly in Group II (9 28±4 65) and demonstrated a pronounced increase in Group III (26 30±4 50) which had the longest post natal survival time For the group of neonates as a whole there was a significant correlation between thyroid iodine concentration and survival time ($r=0\ 61$ $p<0\ 01$)

These changes in total iodine concentration were the resultant of two factors The first was an increase in the thyroglobulin concentration with time values changing from 1 43 mg/100 mg±0 88 in Group I to 1 92±0 77 in Group II and significantly to 3 20±0 20 in Group III ($r=0\ 49$ $p<0\ 05$) However even in Group III thyroids the concentration of thyroglobulin was well below that found in adult glands (9 53±2 83) (unpublished data)

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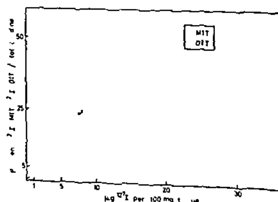


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2	9 5	31	3 25	0 88
3	11 5	32	13 20	2 75
4	16 3	32	11 20	1 87
5	17 0	29	2 75	1 10
Mean±SD			6 25±4 96	1 43±0 88
Groups II	(hours)			
6	26 0	28	7 70	1 10
7	31 0	30	3 45	0 66
8	32 0	30	9 00	1 80
9	34 0	37	17 90	2 60
10	35 0	31	8 00	1 80
11	36 0	30	5 15	2 00
12	38 0	31	15 30	3 30
13	48 0	30	10 30	1 80
14	72 0	30	6 70	2 20
Mean±SD			9 28±4 65	1 92±0 77
Group III	(days)			
15	12	26	21 80	2 90
16	13	36	32 50	3 50
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globulin as well as its iodine and individual iodoamino acid contents in the thyroids of the same neonates

MATERIAL

Thyroid glands from 17 neonates ranging from 26 to 37 weeks in gestational age were obtained at autopsies performed between 2 and 16½ hours following death (mean 10 hours) during which time the specimens were kept at 4 In Table 1 the various thyroid samples are arranged according to the survival time (3 hours 10 minutes to 49 days) Group I includes the thyroid glands of neonates who survived less than 20 hours Group II comprises those with a survival time between 26 and 72 hours and Group III includes the thyroids of neonates who lived for more than 10 days All biochemical analyses were performed in one lobe the other being used for histological examination Lobe weights varied from 210 to 610 mg except in specimen no 1 which weighed 2 g

METHODS

As soon as the thyroid was removed the tissue was frozen at – 20° until used Soluble proteins were obtained by extracting thyroid slices with 0 14 M NaCl (200 mg wet tissue per ml) and centrifuging the extract at 8 000 x g for 20 min in a refrigerated MSE centrifuge The tissue pellet was solubilized with N NaOH and diluted The volumes of both the soluble supernatants and the accompanying pellets were determined [127]I content was measured using a semi automatic micromethod Each sample was digested at 245 for 30 min with a mixture of concentrated sulfonitroperchloric acids After dilution stable iodine was determined using the ceric arsenious reaction in a Technicon Autoanalyser (14) The method is applicable in the range of 1 to 10 ng iodine per sample with a precision of 5%

The protein content of the soluble extract was evaluated by measuring the absorption at 260 and 280 nm (20) and by the method of Folin (16) Values obtained with the two methods aggreed closely

The separation of thyroglobulin from the other soluble proteins was performed by electrophoresis in a 5% polyacrylamide gel using a Pleuger apparatus according to the method of Barka (2) The buffer employed was Tris glycine 0 02 M pH 8 3 An aliquot of the soluble extract containing about 50–100 μg protein and 300 ng iodine was overlaid in 50 μl of 10% sucrose on the top of the gel great care was exercised in applying the sample as stacking gel was not used Electrophoresis was carried out for about 1 hour at a current of 3 mA per tube

Duplicate gels of about 7 cm length were prepared The first gel was cut in a slicer into 1 3 mm pieces Each slice was placed into a calibrated glass tube and dissolved in 0 15 ml 30% H_2O_2 at 48 during 20 hours The dissolved gels were adjusted to a volume of 0 5 ml and the iodine concentration measured in aliquots Blank measurements in solubilized gel alone were nil This technique was employed instead of sucrose density gradient

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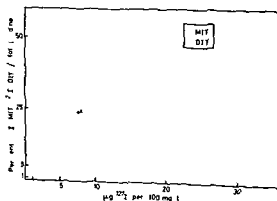


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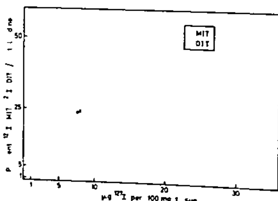


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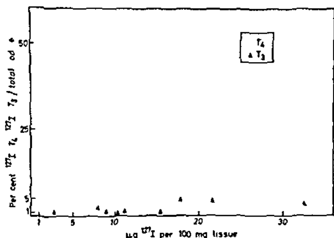


Fig 2 Relationship between thyroid tissues iodine content and hormones percentage in human neonates

increase in the iodine content of thyroglobulin (TG) itself (Table 2) Mean values were 0 16± 0 11 μg ^{127}I/100 μg TG in Group I 0 25±0 11 in Group II and 0 43±0 05 in Group III Owing to the large increase in values found in Group III for the three groups as a whole there was a positive correlation of iodine content in thyroglobulin with neonatal survival time that was of borderline statistical significance ($r=0\ 49$ $p=0\ 05$)

Content of iodoamino acids

When calculated on the basis of their iodine content relative to total iodine the proportions of mono and diiodotyrosine (MIT and DIT) did not vary among the three groups of neonates In the case of MIT percentages ranged between 17 and 30 with a mean of 23±5 In the case of DIT percentages varied between 15 and 41, with a mean of 30±6 The disperson of percentages was slightly larger for DIT than for MIT, the percentages of MIT and that of DIT were not correlated with the iodine concentration of the tissue (Fig 1)

Similarly no marked systematic variation with survival time and hence with thyroid iodine concentration was seen in the fraction of total thyroid iodine contributed by T_4 (Fig 2) values ranging between 18 and 34% of total iodine, with a mean of 27±5

As would be expected from the foregoing relationships, calculated values for the number of residues of each of these three iodinated amino acids increased progressively with increasing duration of survival (Table 2) As judged from analysis of variance, these changes with time were statistically significant (for MIT $p<0\ 01$, for DIT $0\ 01<p<0\ 05$ and for T_4 $p=0\ 01$)

Except in Group III in which clearly measurable values for T_3 were obtained the quantities of iodine in T_3 were near the limit of detectability of the methods employed hence values of T_3 residues should be considered with caution in the case of Groups I and II The data leave no doubt however that an increase in both the proportion and absolute number of residues of T_3 occured in Group III

DISCUSSION

The present studies have provided unavailable information concerning the iodine thyroglobulin and iodoamino acid content of the human newborn in the early hours after birth and of the changes that have occurred after the first few weeks of life There are obvious difficulties encountered in conducting or interpreting a study of this type many of which cannot be surmonted First the studies must obviously be carried out in tissues of babies who have expired and the question could be raised therefore as to the effects of the underlying illness upon the results obtained Although this possibility cannot be excluded the infants from whom tissues had been obtained died of a variety of disorders some spontaneous and some traumatic (8) Moreover there is currently no information that would relate in a causative way the occurrence of a lethal illness and certain of the abnormalities that we have observed In addition the small amount of tissue available and its low iodine content created potential analytic difficulties but these were important only in the case of T_3 analysis in thyroids obtained during the first few hours after birth

A major finding of the present studies is the demonstration that the iodine concentration per unit weight of the thyroid is far lower in the early neonate than it is later in the neonatal period This can be explained in large part by the lower concentration of TG present in the early neonatal thyroid a finding that correlates well with lack of colloid seen on histologic examination as reported earlier (8) This phenomenon can result from the stimulation caused by TSH surge (10 4) few minutes after birth at the end of the TSH peak the thyroidal components gradually increase Most likely the release of the intrathyroidal stores explain the increased concentration of TG present in the cord blood (12) Moreover the role of an enhanced rate of TG proteolysis either antecedent to or coincident with birth is unknown Finally it is possible that the capacity of the fetal thyroid to synthesize thyroglobulin is limited and improves after birth although this cannot be studied

As shown in the analyses of Group III thyroids an increase in TG and iodine concentrations occurs between the first few days and the first few weeks of life Values for thyroid iodine concentration obtained in this group accord well with those previously reported (17) Nevertheless values for iodine concentration even in this group are below those found in the thyroid of the normal adult (6)

The second major finding of the present study relates to the iodine and iodoamino acid content of the TG itself The mean iodine content of TG was below the lower limit of the normal range in Group I thyroids increased into the normal range in Group II thyroids and was at the upper limit of the normal range in Group III thyroids (19) No explanation is available for this increase with increasing survival time in the iodine content of TG

What was most striking among the present results was the pattern of iodoamino acid content *in thyroglobulin* when related to its iodine content It is well known from studies of iodine-depleted rats (15) and of goitrous human thyroids (7) that the low levels of TG iodination are associated with an increased proportion of MIT relative to DIT a decrease in iodothyronine/iodotyrosine ratio and an increase in T_3/T_4 ratio The present studies of TG from neonatal thyroids revealed none of these relationships despite the wide variation in iodine contents of TG seen among the three groups studies Proportions of MIT were not particularly high nor were proportions of T_4 particularly low even in Group I thyroids Possibly the degree of lowering of iodine content in the neonatal thyroid being less severe than that in the TG of the iodine depleted rat or goitrous human thyroid is insufficient to elicit changes in iodoamino content of the type described above

The last puzzling finding is the transiently low content of T_3 found in the thyroid of the neonate *during the first hours of life however* T_4/T_3 ratios from Group I and II are close to the values found in the fetus (11) Ratio from the thyroids of Group III is slightly different if we consider these 3 values are representative of long survival neonates this ratio is lower than that found in the thyroid of adults (3) A similar lack of T_3 has been observed in the fetus and neonatal pigs (9) suggesting that the phenomenon observed in the present study may be general Our findings can be linked to the low concentration of T_3 found in cord blood in the neonates (1) and the T_3 serum increase observed few minutes after birth triggerd by the TSH surge could be due to an accelerated conversion of T_4 to T_3 the intra thyroidal stores are in suffisant amount to provide it The T_4 changes observed later in the serum correspond to the onset of the repleted stores in the thyroid

ACKNOWLEDGEMENTS

The author thanks Miss Françoise Gehin for the excellent technical assistance and is indebted to Dr J C Larroche for supplying *the thyroid specimens and to Dr B* Ransil for statistical analyses She also expresses her gratitude to Dr S H Ingbar for his thorough and critical review of this manuscript

The study was supported by le Centre National de la Recherche Scientifique

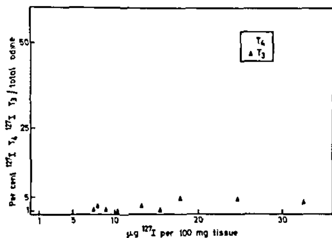


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A major finding of the present studies is the demonstration that the iodine concentration per unit weight of the thyroid is far lower in the early neonate than it is later in the neonatal period This can be explained in large part by the lower concentration of TG present in the early neonatal thyroid a finding that correlates well with lack of colloid seen on histologic examination as reported earlier (8) This phenomenon can result from the stimulation caused by TSH surge (10 4) few minutes after birth at the end of the TSH peak the thyroidal components gradually increase Most likely the release of the intrathyroidal stores explain the increased concentration of TG present in the cord blood (12) Moreover the role of an enhanced rate of TG proteolysis either antecedent to or coincident with birth is unknown Finally it is possible that the capacity of the fetal thyroid to synthesize thyroglobulin is limited and improves after birth although this cannot be studied

As shown in the analyses of Group III thyroids an increase in TG and iodine concentrations occurs between the first few days and the first few weeks of life Values for thyroid iodine concentration obtained in this group accord well with those previously reported (17) Nevertheless values for iodine concentration even in this group are below those found in the thyroid of the normal adult (6)

The second major finding of the present study relates to the iodine and iodoamino acid content of the TG itself The mean iodine content of TG was below the lower limit of the normal range in Group I thyroids increased into the normal range in Group II thyroids and was at the upper limit of the normal range in Group III thyroids (19) No explanation is available for this increase with increasing survival time in the iodine content of TG

What was most striking among the present results was the pattern of iodoamino acid content in thyroglobulin when related to its iodine content It is well known from studies of iodine-depleted rats (15) and of goitrous human thyroids (7) that the low levels of TG iodination are associated with an increased proportion of MIT relative to DIT a decrease in iodothyronine/iodotyrosine ratio and an increase in T_3/T_4 ratio The present studies of TG from neonatal thyroids revealed none of these relationships despite the wide variation in iodine contents of TG seen among the three groups studies Proportions of MIT were not particularly high nor were proportions of T_4 particularly low even in Group I thyroids Possibly the degree of lowering of iodine content in the neonatal thyroid being less severe than that in the TG of the iodine depleted rat or goitrous human thyroid is insufficient to elicit changes in iodoamino content of the type described above

The last puzzling finding is the transiently low content of T_3 found in the thyroid of the neonate during the first hours of life however T_4/T_3 ratios from Group I and II are close to the values found in the fetus (11) Ratio from the thyroids of Group III is slightly different if we consider these 3 values are representative of long survival neonates this ratio is lower than that found in the thyroid of adults (3) A similar lack of T_3 has been observed in the fetus and neonatal pigs (9) suggesting that the phenomenon observed in the present study may be general Our findings can be linked to the low concentration of T_3 found in cord blood in the neonates (1) and the T_3 serum increase observed few minutes after birth trigged by the TSH surge could be due to an accelerated conversion of T_4 to T_3 the intra thyroidal stores are in suffisant amount to provide it The T_4 changes observed later in the serum correspond to the onset of the repleted stores in the thyroid

ACKNOWLEDGEMENTS

The author thanks Miss Françoise Gehin for the excellent technical assistance and is indebted to Dr J C Larroche for supplying the thyroid specimens and to Dr B Ransil for statistical analyses She also expresses her gratitude to Dr S H Ingbar for his thorough and critical review of this manuscript

The study was supported by le Centre National de la Recherche Scientifique

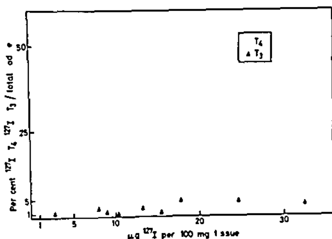


Fig 2 Relationship between thyroid tissues iodine content and hormones percentage in human neonates

increase in the iodine content of thyroglobulin (TG) itself (Table 2) Mean values were 0 16± 0 11 μg ^{127}I/100 μg TG in Group I, 0 25±0 11 in Group II, and 0 43±0 05 in Group III Owing to the large increase in values found in Group III for the three groups as a whole there was a positive correlation of iodine content in thyroglobulin with neonatal survival time that was of borderline statistical significance (r=0 49, p=0 05)

Content of iodoamino acids

When calculated on the basis of their iodine content relative to total iodine the proportions of mono and diiodotyrosine (MIT and DIT) did not vary among the three groups of neonates In the case of MIT percentages ranged between 17 and 30 with a mean of 23±5 In the case of DIT percentages varied between 15 and 41 with a mean of 30±6 The disperson of percentages was slightly larger for DIT than for MIT the percentages of MIT and that of DIT were not correlated with the iodine concentration of the tissue (Fig 1)

Similarly, no marked systematic variation with survival time and hence with thyroid iodine concentration was seen in the fraction of total thyroid iodine contributed by T_4 (Fig 2), values ranging between 18 and 34% of total iodine with a mean of 27±5

As would be expected from the foregoing relationships, calculated values for the number of residues of each of these three iodinated amino acids increased progressively with increasing duration of survival (Table 2) As judged from analysis of variance, these changes with time were statistically significant (for MIT $p<0$ 01, for DIT 0 01$<p<$0 05 and for T_4 p=0 01)

Except in Group III in which clearly measurable values for T_3 were obtained the quantities of iodine in T_3 were near the limit of detectability of the methods employed, hence values of T_3 residues should be considered with caution in the case of Groups I and II The data leave no doubt however that an increase in both the proportion and absolute number of residues of T_3 occured in Group III

DISCUSSION

The present studies have provided unavailable information concerning the iodine, thyroglobulin and iodoamino acid content of the human newborn in the early hours after birth and of the changes that have occurred after the first few weeks of life There are obvious difficulties encountered in conducting or interpreting a study of this type many of which cannot be surmonted First the studies must obviously be carried out in tissues of babies who have expired and the question could be raised therefore as to the effects of the underlying illness upon the results obtained Although this possibility cannot be excluded the infants from whom tissues had been obtained died of a variety of disorders some spontaneous and some traumatic (8) Moreover there is currently no information that would relate in a causative way the occurrence of a lethal illness and certain of the abnormalities that we have observed In addition the small amount of tissue available and its low iodine content created potential analytic difficulties but these were important only in the case of T_3 analysis in thyroids obtained during the first few hours after birth

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KEY WORDS Preterm infants sodium homeostatis tetrahydroaldosterone excretion

Sulyok reported that plasma sodium levels in healthy preterm infants fall during the first 2 weeks of life reaching a minimum level (mean 133 mmol/l) on about the 17th day (21) A negative salt balance was observed during this period Thereafter a positive sodium balance was restored and plasma sodium levels returned to normal It has been generally accepted that these features of sodium homeostasis are caused by the immaturity of the renal tubules (21 22)

The role of aldosterone in the development of sodium homeostasis in preterm infants is not well understood Other investigators have assumed that the low aldosterone secretion rate during the first week of life (23) precluded a significant role for aldosterone in sodium retention during this period Continuous studies for longer periods have not been reported

A study of urinary tetrahydroaldosterone excretion during the first weeks of life in preterm infants is being undertaken in this laboratory A preliminary communication reported elevated tetrahydroaldosterone excretions in twin 14 day-old infants delivered at 29 weeks gestation (8) The values reported (60–225 μg/24 h) were greatly in excess of those found for normal full term infants in this study and by other investigators (13)

In the present report the aldosterone status has been studied in relation to the sodium balance of preterm infants of gestational ages 26–34 weeks

PATIENTS AND METHODS

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All deliveries (except *Case 3*) were spontaneous and premature Infant *3* was born by elective Caesarian section carried out due to concern arising from rapidly rising maternal blood pressure Age was calculated from the mother's last menstrual period and confirmed by clinical examination (5) In general infants received breast milk collected from mothers in the maternity wards Commercial preparations where used were similar in electrolyte content to human milk Details of fluid and milk intake were recorded Twenty four hour specimens of urine were generally collected in plastic paediatric urine bags although in a few cases disposable napkins

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Submıtted Aprıl 26 1976
Accepted July 30 1976

Unıté 30 INSERM
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Acta Pædiatr Scand 66 103–109 1977

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Table 1 *Characteristics of study group*

Case	Sex	Birth weight (g)	Gestational age (weeks)	Minimum weight (g)	Days for birth weight to be regained
1	M	1 060	27	1 000	17
2	M	1 130	29	1 020	14
3	M	1 520	34	1 470	11
4	F	1 200	31 5	1 100	11
5	F	960	31 5	900	11
6	F	920	27	780	15
7	F	1 200	29	1 060	15
8	M	1 500	34	1 400	7
9	M	1 030	26	960	–

were used In a separate study (to be published) we have shown similar steroid excretion patterns in urine collected in a bag for 24 h and an extract prepared by centrifugation of disposable napkins used during the next 24 h period Capillary blood samples were taken by heel puncture

Informed consent was obtained from the parents for execution of detailed sodium balance studies Male infants were selected because of the relative ease of obtaining accurate 24 h urine collections The normal range for plasma sodium concentration in full term infants at this hospital is 135–145 mmol/l and for the purpose of this study hyponatraemia was defined as a persistent concentration below this range

A weight loss after birth was observed in all infants with minimum weights recorded on the 4th or 5th day Birth weights were regained between days 7 and 18 (Table 1) The infants were discharged from hospital when they had reached a weight of 2 5–3 kg and subsequent progress has been uneventful

The prenatal and intrapartum histories of all mothers were closely scrutinised All were on unrestricted salt diets and received no diuretics during the later stages of pregnancy

Analytical methods

Sodium and potassium concentrations were determined simultaneously by twin channel flame photometry Lithium was added to the samples for use as internal standard

Urinary tetrahydroaldosterone was measured by a gas chromatographic mass spectrometric (GC–MS) method (9 16 17) This steroid is excreted in urine as a 3 glucosiduronate and had to be released from conjugation by enzymic hydrolysis (digestive juice of the snail *Helix pomatia*) An internal standard of 3β-*allo* tetrahydroaldosterone was added prior to steroid extraction on Amberlite XAD 2 resin columns The extracts were fractionated on small Sephadex LH 20 columns and methyloxime trimethylsilyl ethers were prepared of the fraction containing tetrahydroaldosterone The derivatives were analysed by GC MS using a Varian MAT 731 instrument in a selective ion monitoring mode The fragment ions of mass to charge ratio 638 (molecular ion M⁺) and 607 (M⁺−31) were alternately monitored at 1 sec intervals

The peak height of the *m/e* 607 ion of urinary tetrahydroaldosterone was measured relative to the peak height of the equivalent ion derived from the internal standard separated by gas chromatography The ion at *m/e* 638 was only monitored to confirm the specificity of the determination The results were calculated according to the formula

$$X = \frac{H_X}{H_S} \times R \times \frac{V}{U} \times S$$

X = Urinary tetrahydroaldosterone excretion (μg/24 h)
H_X = Peak height urinary tetrahydroaldosterone

H_S = Peak height 3β-*allo*-tetrahydroaldosterone

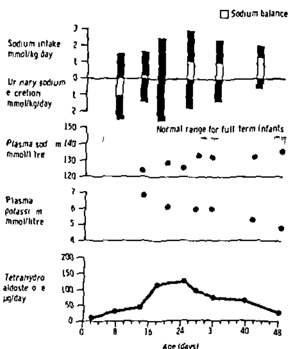


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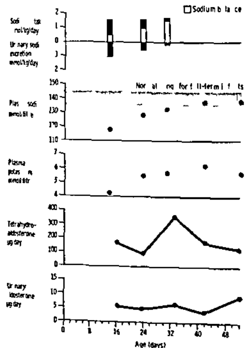


Fig 2 Postnatal values of sodium intake and excretion plasma electrolyte concentrations and urinary tetrahydro-aldosterone in a preterm infant delivered at 29 weeks gestation (*Case 2*) The urinary excretion of aldosterone is the sum of free aldosterone and aldosterone 18 glucosiduronate

S =Weight of internal standard (µg)
R =Response factor=H_S / H_X

H_X $H_S{}^1$=Calibrating standards Peak heights of identical weights of reference tetrahydroaldosterone and 3β *allo*-tetrahydroaldosterone respectively
V =24 h urine volume (ml)
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Urinary aldosterone (free and 18 glucosiduronate) was measured by radioimmunoassay following pH 1 hydrolysis and chromatography (12)

RESULTS

Fig 1 illustrates the results of the detailed investigation of a male infant (*Case 1*) delivered at 27 weeks gestation On the 8th day of life urinary sodium excretion exceeded dietary intake by 1 mmol/kg/day A positive sodium balance was achieved by the 25th day of life Little significance can be attributed to the small positive balance observed on day 14 since in this study sodium loss other than in urine (e g faeces or sweat) were not determined Potassium intakes were around 3 2 mmol/kg/day and during the first 5 weeks of life potassium excretion did not exceed 1 6 mmol/kg/day with positive balances of around 2 0 mmol/kg/day On the 40th day potassium excretion rose to 2 6 mmol/kg/day and the ratio of urinary concentration of sodium to potassium fell to 0 46 from 1 30 on day 32 This infant was hyponatraemic and hyperkalaemic during the first 4 weeks of life thereafter the plasma sodium and potassium concentrations approached normal levels for full term infants The infant regained birth weight by day 17 Tetrahydroaldosterone excretion during the first 15 days of life remained within the upper limits of normal for a full term infant A significant rise was recorded between days 14 and 18 and levels remained high for 2–3 weeks

Figs 2 and 3 show similar data for infants delivered at 29 and 34 weeks gestation (*Case 2 and 3*) In both these patients positive sodium balance was observed by day 14 Unfortunately sodium balance studies and urinary tetrahydroaldosterone excretions were

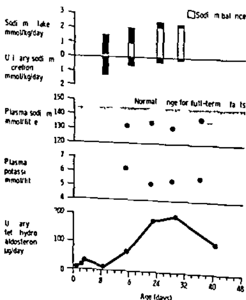


Fig 3 Postnatal values of sodium intake and excretion plasma electrolyte concentrations and urinary tetrahydroaldosterone in a preterm infant delivered at 34 weeks gestation (*Case 3*)

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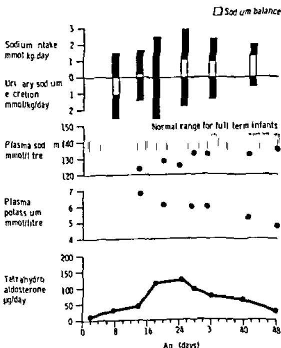


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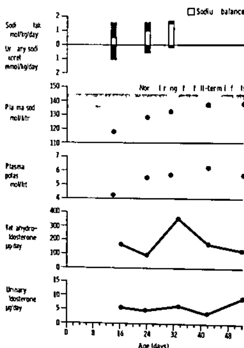


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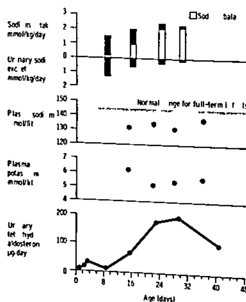


Fig 3 Postnatal values of sodium intake and excretion plasma electrolyte concentrations and urinary tetrahydroaldosterone in a preterm infant delivered at 34 weeks gestation (*Case 3*)

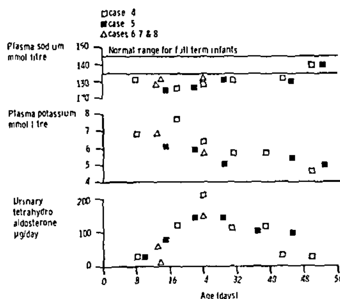


Fig 4 Urinary tetrahydroaldosterone excretion and plasma electrolyte concentrations in relation to postnatal age of 5 preterm infants

not obtained for *Case 2* during the first 2 weeks of life A normal daily intake of potassium for infant 2 was 2 7 mmol/kg/day Potassium balances on days 16 24 and 32 were 0 6 2 1 and 1 2 mmol/kg/day An increase in daily excretion of potassium was not observed but the ratio of sodium to potassium concentration in urine fell from 0 48 on day 16 to 0 09 at day 30 Urinary aldosterone excretions were determined on this patient and the levels were higher than those found for normal infants studied in this laboratory (1 to 2 µg/24 h) Plasma sodium concentrations increased during the period of study In one of the patients (*Case 2*) the plasma potassium concentration appeared also to increase but on this occasion results may have been unreliable because of haemolysis

Results of plasma electrolyte and tetrahydroaldosterone excretions for several other infants are illustrated in Fig 4 Since urinary extracts were occasionally obtained from disposable napkins it was not possible to determine sodium balance because urinary sodium was not assayed Tetrahydroaldosterone excretion in *Cases 4* and *5* show a similar pattern to that of the infants described above Values obtained during the first 10 days were low but a significant increase was observed after 15th day The plasma sodium concentrations were low in both infants during the first 6 weeks of life but approached normal values by the 8th week The trend of plasma potassium levels was reversed

Cases 6 7 and *8* were only studied for one day but the results were comparable to those obtained at the same age for the infants studied more extensively

Daily sodium balance studies and urinary tetrahydroaldosterone excretions were obtained for an infant born at 26 weeks gestational age (*Case 9*) The results obtained are illustrated in Fig 5 and demonstrate a severe negative balance throughout the study Sodium intake includes the sodium content of all fluids administered over the 24 h period Fluid injected intravenously varied considerably in volume and between the 5th and 8th day of life were particularly high Tetrahydroaldosterone excretion was extremely low during the first week of life and did not respond to the negative salt balance An increase was observed in the 2nd week although the high excretions ob

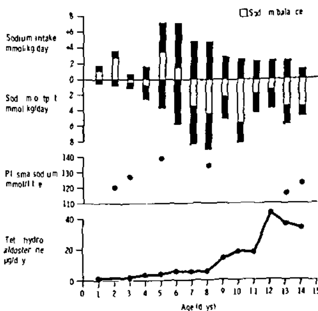


Fig 5 Daily changes in sodium intake and excretion in relation to plasma sodium concentrations and urinary tetrahydroaldosterone excretion The infant (*Case 9*) was delivered at 26 weeks gestation

tained in the other cases were not achieved This increased secretion of aldosterone was evidently insufficient to arrest the sodium loss and death occurred on the 15th day of life

DISCUSSION

Aldosterone can be synthesised by the foetal adrenal from early in gestion Dufau & Villee (6) achieved a synthesis of aldosterone from progesterone by adrenal homogenates from foetuses 15 weeks old and Pasqualini et al (16) demonstrated that perfusion of a 20 week old foetus with labelled corticosterone resulted in aldosterone synthesis It may therefore be concluded that viable preterm infants are capable of aldosterone synthesis A significant amount of aldosterone circulating in the full term foetus at birth is of foetal origin and relatively high levels persist in the newborn for several days (2 3)

Aldosterone catabolism is considered to be similar in fullterm neonates and adults since New and co-workers (13) found comparable proportions of urinary free aldosterone its 18 glucosiduronate and tetrahydroaldosterone glucosiduronate However the problems of determining aldosterone production are exacerbated when preterm infants are being studied since metabolising enzymes in the liver and kidney may be immature resulting in altered steroid catabolism Premature infants aged 33–75 days appear to excrete a disproportionately high amount of free aldosterone compared to the other aldosterone metabolites (13) The high tetrahydroaldosterone excretions demonstrated in the present study provide *in vivo* evidence for the capacity of preterm infants at a developmental age of 28 weeks gestation to catabolise aldosterone in a similar manner to an adult In one of our patients (*Case 2*) aldosterone (free plus 18 glucosiduronate) was determined in urine as well as tetrahydroaldosterone The ratio of tetrahydroaldosterone to aldosterone was generally greater than 20 1 which compares with a ratio of about 2 1 for the full term newborns studied by New et al (13) However our results are not directly comparable with those of New and co-workers who studied newborns only during the first 24 h of life when urine may include metabolites of maternal aldosterone Although the ratio of the individual aldosterone metabolites excreted by preterm infants full term infants and adults appears to be at variance there is no doubt that tetrahydroaldosterone is the major metabolite of aldosterone in preterm infants and must reflect aldosterone secretion

The urinary tetrahydroaldosterone excretion during the first week of life in premature infants were generally slightly higher than those found for full term neonates (14 19) and increased considerably around the 15th day Although the early tetrahydroaldosterone excretions are higher than adults when adjusted for body surface area the increase in aldosterone secretion suggests a transient period of mineralocorticoid deficiency after birth

The high renin activity in the first weeks of life of full term neonates reported by Kotchen et al (10) suggests a lower responsiveness of aldosterone synthesis to renin angiotensin stimulation during this period and may explain why aldosterone secretion is not significantly stimulated by hyponatraemia when associated with the presence of a negative salt balance Siegel et al (19) found a high level of plasma aldosterone in preterm infants during the first few days of life and state that this finding precludes aldosterone insufficiency being responsible for the sodium wasting and hyponatraemia observed If their patients had been studied for a longer period even higher plasma aldosterone levels would almost certainly have been found and their conclusions invalidated These authors have assumed that high aldosterone levels are synonomous with high activity High plasma aldosterone concentrations may reflect a compensation for lower response of the renal tubules to the hormone or are required to counteract an aldosterone antagonist The newborn synthesises many

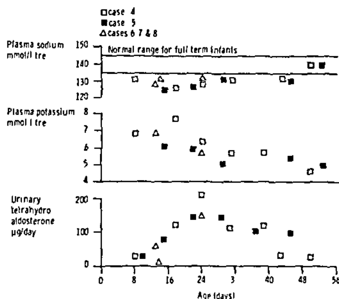


Fig 4 Urinary tetrahydroaldosterone excretion and plasma electrolyte concentrations in relation to postnatal age of 5 preterm infants

not obtained for *Case 2* during the first 2 weeks of life A normal daily intake of potassium for infant 2 was 2 7 mmol/kg/day Potassium balances on days 16, 24 and 32 were 0 6 2 1 and 1 2 mmol/kg/day An increase in daily excretion of potassium was not observed but the ratio of sodium to potassium concentration in urine fell from 0 48 on day 16 to 0 09 at day 30 Urinary aldosterone excretions were determined on this patient and the levels were higher than those found for normal infants studied in this laboratory (1 to 2 µg/24 h) Plasma sodium concentrations increased during the period of study In one of the patients (*Case 2*) the plasma potassium concentration appeared also to increase but on this occasion results may have been unreliable because of haemolysis

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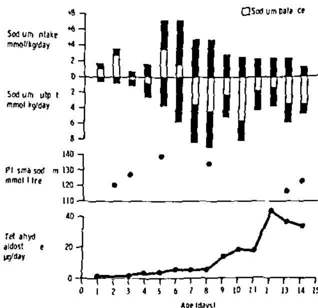


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Submitted April 20 1976
Accepted July 30 1976

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steroids in large amounts which may antagonise the action of aldosterone on the renal tubules For example 16α hydroxyprogesterone has a natriuretic effect in adults (11) and while this particular steroid may not be responsible for opposing the activity of aldosterone in newborns many similar steroids are secreted by the adrenals in large amounts

It is not known whether the increased aldosterone secretion in the third week of life reflects a response to increased stimulation or to improvement in the ability of the adrenals to synthesise larger amounts The renal tubules are assumed to respond to aldosterone at this stage since a positive sodium balance is established with the consequent concentrations of sodium and potassium in plasma However the renal tubules cannot be responding optimally to aldosterone since sodium is still excreted in urine when plasma sodium concentration remains low and a direct renal exchange of sodium with potassium was not demonstrated until the 6th week of life Insufficient sodium reabsorption may be due to earlier maturation of renal glomeruli resulting in an imbalance between tubule and glomerular mass (7) Aperia et al (1) consider that the relatively high fractional sodium excretion in preterm infants may be attributed to an inadequate tubular surface area These investigators concluded that aldosterone was not important in tubular sodium reabsorption on the basis of the low aldosterone secretion rates reported by Weldon and co workers (23)

In spite of sodium retention the aldosterone secretion may subsequently continue to be high in response to the plasma sodium concentration remaining below normal or to hypertrophy of the *zona glomerulosa* The sodium balance figures reflect largely the amount of sodium used in growth From the third week of life the figures observed (1–2 mmol/kg/day) agree with the rates of accumulation of sodium by human foetuses in relation to gestation (18)

Further studies involving determinations of aldosterone metabolism and renin activity are required to resolve the outstanding problems of sodium regulation in infancy A method for the analysis of renin activity in small amounts of blood has recently been published by Dillon (4) and determinations of renin activity using this method are to be included in future investigations

ACKNOWLEDGEMENTS

We are indebted to Miss D Davis and the nursing staff of the Special Care Baby Unit Northwick Park Hospital for their co-operation in these studies The determinations of urinary aldosterone were kindly carried out by C E Horth of Searle Diagnostics

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Acta Pædiatr Scand 66 111–116 1977

PLASMA TRIGLYCERIDE INCREASE AFTER ORAL FAT LOAD IN MALABSORPTION DURING EARLY CHILDHOOD

S P FALLSTRÖM C O NYGREN and R OLEGÅRD

From the Department of Paediatrics I East Hospital University of Goteborg Sweden

ABSTRACT Fallstrom S P Nygren C-O and Olegård R (Department of Paediatrics I East Hospital University of Goteborg Sweden) Plasma triglyceride increase after oral fat load in malabsorption during early childhood Acta Paediatr Scand 66 111 1977.—Plasma triglyceride (TG) levels were determined before and after a test meal of 2 g neutral fat per kg b w in 32 children with different malabsorption syndromes 8 children with transient growth retardation without demonstrable disease and in 29 controls The increase of plasma TG 120 min after the test meal was found to discriminate between children with malabsorption syndromes and controls during the studied age period 1 month–3 years In this respect it was superior to faecal fat determination Children with transient growth retardation who later spontaneously regained satisfactory growth although along a lower SD score than the initial one showed a TG increase after the test meal comparable to that of the controls

KEY WORDS Fat absorption test malabsorption coeliac disease cystic fibrosis growth retardation children

Utilisation of dietary fat requires digestion in the presence of bile salts and lipases at a suitable pH and transport through the instestinal mucosa Disturbed fat absorption occurs in several gastrointestinal diseases and determination of the fat absorption has become a valuable diagnostic tool It is usually accomplished by determination of the fat content in faeces collected during three or more days with the patient on a standardized diet the fat content in faeces often expressed as a fraction of consumed fat (2 6) However in small children quantitative collection of faeces during several days is laborious and in case of diarrhoea difficult to achieve Furthermore during infancy the coefficient of fat absorption varies with age and type of feeding (3 15) which must be considered in the evaluation of faecal fat content Under certain conditions the blood concentration of a lipid substance after a test meal containing the lipid in case is a measure of its absorption (10) Chylomicrographic and turbidometric estimation of the lipaemia after a test meal of neutral fat has been used mainly in experimental work (4 5 13) but because of methodological shortcomings (14) these methods have found little application in clinical work

Nowadays simple and accurate spectrophotometric methods for determination of the triglyceride (TG) concentration in plasma are available in most hospitals Therefore we have tested the value of plasma TG levels after oral fat load in the diagnosis of malabsorption in early childhood

MATERIAL

The following groups of children were studied

Controls Twentynine children aged 3 weeks to 20 months—14 of whom were less than 4 months of age without gastrointestinal symptoms and signs of infection All showed satisfactory growth and weight increase at the time of study and continued to do so at follow up controls later

Glutenenteropathy Twentyfive children aged 5 to 21 months with longstanding diarrhoea and/or failure to

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None of the controls older than four months had a TG increase at 2 hours less than 0 50 mmol/l and none of the children of that age with defined clinical malabsorption an increase exceeding 0 75 mmol/l (Table 1) Thus after 4 months of age an increase less than 0 50 mmol/l can be regarded as abnormal and values between 0 50 and 0 75 mmol/l as borderline Some of the controls younger than four months of age showed a less efficient fat absorption but their TG increase at 2 hours after the test meal always exceeded 0 30 mmol/l (Table 1) In the pathological groups which usually have to be considered during this early period i e infants with cystic fibrosis or hepatobiliary disease the increase was always poorer

Thus using a TG increase in plasma of 0 30 mmol and 0 50 mmol/l respectively at 2 hours after a test meal as the lower limits accepted for healthy infants before and after 4 months of age 30 out of 32 (94%) of children with different malabsorption syndromes were found to have abnormal triglyceride absorption The two remaining pathological cases fell in the borderline range but showed a lower percental increase than the age matched controls

Norman et al (8) found that in healthy infants the TG increase after a test meal exceeded 60% of the fasting level a value never reached by their pathological cases According to the present results this criterion is applicable after the age of four months while before that age a TG increase of 40% seems to be a better discriminating level Using the percental TG increase gave the same degree of overlapping between controls and children with glutenenteropathy as the absolute increase but different cases fell in the borderline range This depends on the fact that in glutenenteropathy not only the absolute TG increase but also the fasting TG level differs significantly from that of the controls Analysis of the relation between TG fasting level and TG increase at 2 hours indicates that at a fasting level below 0 90 mmol/l an absolute increase of 0 50 mmol per l is a preferable critical level while at higher fasting level a percental increase of 60% gives the best discrimination

The discriminating capacity of the TG absorption test compares favourably with that of the xylose test or the faecal fat determination (Table 3) It is especially noteworthy that the oral fat absorption test is superior to the much more time consuming and labourious faecal fat determination

In the group with so called transient growth retardation clinical and laboratory investigations did not reveal any gastroenterologic disease or other pathologic condition The result of the TG absorption test showing a TG increase equal to that in the age matched controls is in accordance with these findings The subsequent clinical course with spontaneous regain of adequate growth following a new growth SD score suggests that these children represent a normal variant of growth pattern (7)

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Table 3 *Proportion of pathological results with the xylose test faecal fat determination and TG absorption test in glutenenteropathy*

Xylose	Faecal fat	TG absorption
19/25 (76%)	11/20 (55%)	23/25 (92%)

Using a TG increase at 2 hours of 0 50 mmol/l as discriminating level

of the fasting level In three of these infants the TG increase was less than 0 50 mmol per l and in another three infants between 0 50 and 0 75 mmol/l (Table 2) The percental increase was 44, 48 and 59% and 74 78 and 144% respectively There was no significant regression of the absolute or percental TG increase at 2 hours after the test meal upon age in the control group (r=0 01 and −0 21)

In children with glutenenteropathy the fasting TG level was significantly higher (p<0 01) than in the age matched controls but no or only slight increase of the TG level was found at 2 hours A delayed but moderate increase was found at 4 hours in 14 out of the 25 cases (Fig 1) The difference between the level at 2 hours and the fasting level varied between −0 31 and 0 73 mmol per l (−26 and 62 mg/100 ml) (Table 1 Fig 2) In the two cases with an increase exceeding 0 50 mmol/l the percental increases were 45 and 55% (Table 2) The percental increase was less than 58% of the initial value in all cases but one in which a low absolute increase of 0 42 mmol/l corresponded to a percental increase of 97% In patients with defective digestion the test meal gave only minute plasma TG changes, the TG increase at 2 hours never exceeding 0 20 mmol/l or 23% of the fasting level In contrast children with transient growth failured showed a TG increase of the same magnitude and with the same pattern as the controls (Fig 1–2 Table 1–2)

In Table 3 the TG absorption test the xylose test and the determination of faecal fat are compared with regard to their discriminating capacity in glutenenteropathy

DISCUSSION

In the present study the triglyceride (TG) level in plasma after a test meal was determined in connection with an analysis of the plasma fatty acid pattern, the results of which will presented in a subsequent paper The TG concentration was therefore calculated from a gas liquid chromatographic determination of the fatty acid pattern by the use of an internal standard As mentioned above the TG values obtained by this method are interchangeable with those determined by spectrophotometric methods in common use (9) Thus a TG absorption test as described here can easily be applied in clinical routine

In the controls a substantial increase of the plasma TG had occurred at 2 hours after the test meal while at 4 hours a return to or towards the fasting level had taken place in most of the children (Fig 1) In healthy adults the peak concentration of plasma lipids after a test meal has been reported to appear later appr after 4 hours and the increased level to be sustained longer (4 11) In these investigations on adults 1 5 g or less of butterfat per kg b w was given and the above mentioned differences may depend on the amounts and types of fat used (15) the Flora® margarine containing much more unsaturated fatty acids The possibility of physiological differences between young children and adults must be considered implying a more rapid fat resorption and utilisation in the former

Most controls younger than 4 months presented a TG increase after the test meal comparable to that in the older controls but in three of the younger infants the increase was smaller (Fig 1 2 and Table 2) This is in agreement with the reports on a less efficient fat absorption in very young infants (3 8) but the close relationship in healthy infants between age and TG increase after a test meal reported by Norman et al (8) could not be confirmed in the present study

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Using a TG increase at 2 hours of 0 50 mmol/l as discriminating level

of the fasting level In three of these infants the TG increase was less than 0 50 mmol per l and in another three infants between 0 50 and 0 75 mmol/l (Table 2) The percental increase was 44 48 and 59% and 74 78 and 144% respectively There was no significant regression of the absolute or percental TG increase at 2 hours after the test meal upon age in the control group (r=0 01 and −0 21)

In children with glutenenterophaty the fasting TG level was significantly higher (p<0 01) than in the age matched controls but no or only slight increase of the TG level was found at 2 hours A delayed but moderate increase was found at 4 hours in 14 out of the 25 cases (Fig 1) The difference between the level at 2 hours and the fasting level varied between −0 31 and 0 73 mmol per l (−26 and 62 mg/100 ml) (Table 1 Fig 2) In the two cases with an increase exceeding 0 50 mmol/l the percental increases were 45 and 55% (Table 2) The percental increase was less than 58% of the initial value in all cases but one in which a low absolute increase of 0 42 mmol/l corresponded to a percental increase of 97% In patients with defective digestion the test meal gave only minute plasma TG changes the TG increase at 2 hours never exceeding 0 20 mmol/l or 23% of the fasting level In contrast children with transient growth failured showed a TG increase of the same magnitude and with the same pattern as the controls (Fig 1–2 Table 1–2)

In Table 3 the TG absorption test the xylose test and the determination of faecal fat are compared with regard to their discriminating capacity in glutenenteropathy

DISCUSSION

In the present study the triglyceride (TG) level in plasma after a test meal was determined in connection with an analysis of the plasma fatty acid pattern, the results of which will presented in a subsequent paper The TG concentration was therefore calculated from a gas liquid chromatographic determination of the fatty acid pattern by the use of an internal standard As mentioned above the TG values obtained by this method are interchangeable with those determined by spectrophotometric methods in common use (9) Thus a TG absorption test as described here can easily be applied in clinical routine

In the controls a substantial increase of the plasma TG had occurred at 2 hours after the test meal while at 4 hours a return to or towards the fasting level had taken place in most of the children (Fig 1) In healthy adults the peak concentration of plasma lipids after a test meal has been reported to appear later appr after 4 hours and the increased level to be sustained longer (4 11) In these investigations on adults *1 5* g or *less of butterfat per* kg b w was given and the above mentioned differences may depend on the amounts and types of fat used (15) the Flora® margarine containing much more unsaturated fatty acids The possibility of physiological differences between young children and adults must be considered implying a more rapid fat resorption and utilisation in the former

Most controls younger than 4 months presented a TG increase after the test meal comparable to that in the older controls but in three of the younger infants the increase was smaller (Fig 1 2 and Table 2) This is in agreement with the reports on a less efficient fat absorption in very young infants (3 8) but the close relationship in healthy infants between age and TG increase after a test meal reported by Norman et al (8) could not be confirmed in the present study

Patients with digestive insufficiency or glutenenteropathy displayed a slow and min

Acta Pædiatr Scand 66 117–119 1977

CASE REPORT

NEONATAL ACIDOSIS ASSOCIATED WITH TRANSIENT METHYLMALONICACIDURIA AND VITAMIN B12 DEFICIENCY

A J WILLIAMS and J T IRELAND

From the Department of Child Health University of Liverpool and Department of Biochemistry Alder Hey Children s Hospital Eaton Road Liverpool England

ABSTRACT Williams A J and Ireland J T (Department of Child Health University of Liverpool and Department of Biochemistry Alder Hey Children s Hospital Liverpool England) Neonatal acidosis associated with transient methylmalonicaciduria and vitamin B 12 deficiency Acta Paediatr Scand 66 117 1977.—Investigation of a neonate presenting with a metabolic acidosis vomiting and an apnoeic attack revealed abnormal urinary excretion of methylmalonic acid (MMA) associated with a low serum vitamin B12 Restriction of dietary protein was followed by normalisation of acid base balance Reintroduction of normal daily protein intake did not precipitate further acidosis or increased excretion of MMA The transient methylmalonicaciduria was probably due to deficiency of vitamin B12

KEY WORDS Neonate acidosis methylmalonicaciduria vitamin B12 deficiency

The urinary excretion of abnormal amounts of methylmalonic acid (MMA) was reported first in association with vitamin B12 deficiency in adults (1 3) Congenital methylmalonicaciduria was subsequently described (9) and recognised to be due to disorders of methylmalonyl coenzyme A racemase (EC 5 1 99 1) (5) methylmalonyl CoA mutase (EC 5 4 99 2) (7) or to defective synthesis of the coenzyme 5 -deoxyadenosylcobalamin (11) which will secondarily impair methylmalonyl-CoA mutase activity A further disorder of vitamin B12 metabolism involving defective synthesis of methylcobalamin in addition to 5 -deoxadenosylcobalamin has also been reported (6) In those types associated with defective vitamin B 12 metabolism the biochemical abnormality is responsive to treatment with B 12 and the clinical course is usually milder (10)

CASE REPORT

Baby C was born to a primagravida mother by normal delivery at an estimated gestational age of 36 weeks with a birth weight of 3160 g The pregnancy had been unremarkable At birth the baby s condition was satisfactory Apgar score 8 at 1 min No abnormality was noted on routine examination Feeding was initially by gavage with expressed breast milk and Ostermilk Complete Formula Twitching of the limbs was noted on the second day and ascribed to hypocalcaemia (serum calcium 6 4 mg/dl) and hypomagnesaemia (serum magnesium 1 6 mg/dl) Despite calcium and magnesium supplementation and change of feed to Scientific Milk Adaptation (there being insufficient expressed breast milk) the twitching continued Cerebrospinal fluid was normal The twitching became worse on the fourth day and the infant had an apnoeic attack lasting about 30 sec followed by two vomits Subsequently she developed a metabolic acidosis unaccompanied by ketosis Urine pH remained between 4 0 and 4 5 The blood pH remained low at 7 05 for 7? hours during which time further investigation was undertaken Gas chromatographic analysis of a random urine specimen sent for screening purposes showed a large quantity of MMA (greater than 3400 μmol/l) No hyperglycinaemia was demonstrated

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Submitted Febr 18 1976
Accepted July 30 1976

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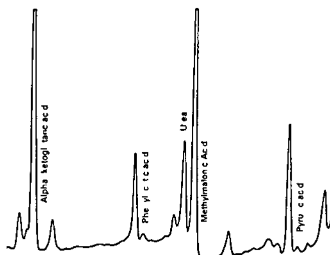


Fig 1 Gas liquid chromatogram First 24 hour specimen showing abnormal excretion of methylmalonic acid

and normal amounts of glycine were detected in the urine In view of the former finding dietary protein was with held and clear fluid feeds given There was dramatic relief of symptoms and of acidosis Analysis of a 24-hour urine collection on the following day confirmed the pre sence of excess MMA (Fig 1) though already the quant ity was markedly reduced the concentration of MMA being equivalent to a 24 hour output of 85 μmol This is outside normal limits (up to 42 5 μmol per day) After the acidosis corrected dietary protein was reintroduced at 0 5 g/kg body weight/day Thereafter in spite of increases in total protein intake no excess of MMA could be de tected in the urine The gas chromatogram of a 24 hour urine taken seven days after initial protein restriction with the infant on a protein intake of 1 g/kg/day and demonstrating a reduction of MMA excretion to normal is shown in (Fig 2) Acid base balance remained normal on a normal protein intake of 3 g/kg/day with no increase in MMA excretion This was attained at age 21 days and the infant was thriving in every way At no time was there any evidence of megaloblastic anaemia

DISCUSSION

Methylmalonicaciduria presenting in the ne onatal period is usually associated with a de vastating illness which has a significant mor tality Restriction of dietary protein alleviates acidosis though we were surprised to find no exacerbation with reinstitution of increasing amounts of protein The absence of ketosis and hyperglycinaemia together with the gen erally mild course of our patient and very modest MMA excretion were also factors suggesting that this was not congenital methyl malonicaciduria as previously described (9)

In children with a methylmalonyl CoA mutase defect or an inborn error of cobalamin metabolism one expects to see daily MMA ex cretion of 500 to 5 000 mg (4) though there has been one report of a child with homocystinuria and a defect in cobalamin metabolism in which MMA excretion was low (8) Our patient had rather high urinary levels of methionine and no increase in homocysteine excretion there by excluding this possibility The serum vita min B12 level done on day eight was 100 picog/ ml and although normal values at this age are ill defined this must be regarded as a low result (2) As the infant had already had by this time some artificial milk feeds containing B12 one might speculate that the cord blood B12 may have been even lower The mother s serum B 12 was 180 picog/ml which was out side the lower limit of normal for the labora tory There was no evidence for present or past maternal megaloblastic anaemia Three weeks after the initial B 12 estimation the baby s serum level had risen to 200 picog/ml

We are unaware of any evidence to suggest transient immaturity of the enzyme systems involved in the conversion of propionate and methylmalonate to succinate The mechanism producing the temporary and mild metabolic acidosis in our patient is probably vitamin B12 deficiency compromising the synthesis of the coenzyme 5 deoxyadenosylcobalamin and thus producing an accumulation of methyl malonate Even when artificial feeds were in troduced the occasional vomits may have led to inadequate vitamin B12 intake With the cessation of vomiting and gradual reintroduc

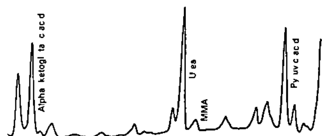


Fig 2 Gas liquid chromatogram 24 hour specimen col lected six days after Fig 1 Showing normal organic acid pattern

tion of dietary protein vitamin B12 levels increased sufficiently to ensure an adequate supply of 5 deoxyadenosylcobalamin The deficiency of vitamin B12 may have been secondary to relative maternal deficiency However cord B12 levels are usually higher than maternal levels at term (2) It is therefore possible that derangement of the B12 placental transport mechanism might account for the abnormal mother/infant B12 ratio seen here

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Submitted April 27 1976
Accepted July 30 1976

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Acta Pædiatr Scand 66 121–125 1977

CASE REPORT

MEDIUM CHAIN TRIGLYCERIDES FOR TREATMENT OF SPONTANEOUS NEONATAL CHYLOTHORAX

Lipid Analysis of the Chyle

B PEITERSEN and B BROCK JACOBSEN

From the University Clinic of Paediatrics Fuglebakken Copenhagen Denmark

ABSTRACT Peitersen B and Jacobsen B B (University Clinic of Paediatrics Fuglebakken Copenhagen Denmark) Medium chain triglycerides for treatment of spontaneous neonatal chylothorax Acta Paediatric Scand 66 121 1977.—Volume and contents of lipid and protein in the pleural fluid from a three weeks old girl with spontaneous chylothorax were studied (*a*) during parenteral nonfatty nutrition and later (*b*) during administration of a formula (Biosorbin®) containing medium chain triglycerides (MCT) The pleural fluid production could not be correlated to the treatment employed but suddenly ceased after 20 days management Triglyceride and total esterified fatty acid concentrations in pleural fluid were high on admission when feeded with human milk and chylomicrons and other lipoproteins were present in the chyle During parenteral treatment a pronounced decrease in pleural fluid concentrations of triglyceride and total fatty acids occurred concomitant with a disappearance of the chylomicrons During the following MCT diet a pronounced increase in triglyceride and total fatty acids concentrations appeared and the chylomicrons reappeared in the chyle The cholesterol and phospholipid concentrations in the pleural fluid showed only small changes during the different treatments No significant changes in protein and albumin concentrations of chyle were observed It is concluded that administration of the Biosorbin® MCT formula containing 87½% of the fat as MCT seems without value in the treatment of spontaneous neonatal chylothorax

KEY WORDS Spontaneous chylothorax medium chain triglycerides

Chylothorax is a serious complication in childhood mainly found in connection with thoracotomy thoracic trauma or tumors (13 19 20)

In the neonatal period spontaneous chylothorax occurs with no clear evidence of the aetiological factors (19 23 26) The usual assumption seems to be that congenital abnormities in the lymphatic system are the main cause (13 19)

Symptoms of chyloaccumulation in the pleural cavity may appear during the first weeks of life (4 19 23 26) The pleural effusion often persists for several weeks but can diminish spontaneously in a few weeks (4 18 19 26) During the last ten years the treatment of various diseases in the lymph system with medium chain triglycerides (MCT) has been acknowledged (10 21) Contrary to more long chained triglycerides MCT is transported directly into the portal system (12 21) bypassing the lymphatic pathways The conclusion has therefore been that a MCT diet would diminish the volume of lymph

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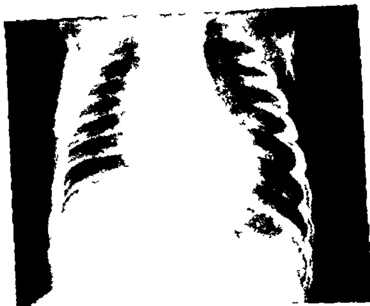


Fig 2 Chest X ray 2 month later completely normal

frozen until analysis Cholesterol (22) phospholipid (6) total fatty acids (8) and triglycerides (15) were determined later Lipoproteinelectrophoresis was performed according to Widme (25) and Frederickson (7) Finally analysis of total *protein* (1) and albumin (14) was made

RESULTS

Amount of lipids in pleural fluid is shown on Table 1 On *admission* to hospital when the child was given mother s milk the concentration of triglycerides and total esterified fatty acids was high compared with values measured later on during the treatment Lipoproteinelectrophoresis showed chylomicrons and other lipoproteins Under administration of the *parenteral* nonfatty treatment the pleural concentration of triglycerides and total fatty acids showed a distinct decrease and chylomicrons could no longer be detected During the following *MCT treatment* the pleural fluid again showed a pronounced increase in the triglyceride and total fatty acids concentration and chylomicrons reappeared The phospholipid and cholesterol concentrations showed only minimal changes during the different periods

No specific changes in the concentration of protein and albumin could be detected

During the MCT diet serumconcentrations of triglycerides cholesterol phospholipids and total fatty acids showed a falling tendency (Table 3) The serum albumin and hemoglobin concentration dropped as long as the pleural effusion persisted and blood transfusions were performed repeatedly

DISCUSSION

The clinical picture and the pleural fluid composition in our patient conform with criteria for a diagnosis of spontaneous neonatal chylothorax (4 13 19 26) It is a well known fact that pleural exudations from chylothorax patients are affected by change in lipidcontent of the diet (2 5 18 24)

Apparently only one report on MCT treatment of spontaneous neonatal chylothorax

Table 2 *Composition of Biosorbin per 100 g*

Supplement of vitamines and minerals in daily required amounts

Total calories		500
Protein	19 9 g	15% of calories
Carbohydrates	60 0 g	50% of calories
Fat	15 2 g MCT 2 5 g essential fatty acids	

80% of the essentiel fatty acids consist of long chained fatty acids



Fig 1 Chest X ray on admission to hospital shoving complete opacification of right hemithorax

through the thoracic duct (9 10 11 21) Three cases of chylothorax in childhood treated with MCT have been reported (3 9 16) and only one had spontaneous neonatal chylothorax

CASE REPORT

The patient is a 3 week-old girl born 3 weeks before term after a pregnancy and birth without any complications Birthweight was 2750 g The child was referred to hospital suffering from increasing respiratory difficulties the past two weeks On arrival she showed symptoms of severe respiratory distress and universal cyanosis Thorax X ray revealed a massive blurring in the right side of thorax and a distinct thrust of trachea to the left (Fig 1)

Intubation and ventilation in respirator were promptly performed and a permanent thoraxdrain was applied which at once removed about 250 ml milky pleural fluid where upon respiration soon became normal The pleural fluid contained numerous lymphocytes but no bacteria and the Sudan IV stain showed plenty of lipids The amount of fluid varied a great deal from day to day through out the whole period but perhaps varying function of the drain accounts for this The pleural effusion ceased completely after 14 days of MCT treatment The amount of chyle removed by drainage averaged 1250 ml The thorax drainage was frequently obstructed and had to be changed seven times before final removal after 24 days The first seven days the patient was treated with parenteral fluids (Table 1) and antibiotics

On day eight a MCT diet was instituted The Biosorbin® formula was used because we expected a long term treatment The composition of Biosorbin is seen from Table 2 Initially was given 80–85 g daily gradually increasing to 100–105 g After three month supplement of fruit vegetables meat and skimmed milk was added and Biosorbin® was discontinued after 6 month X rays of thorax normalized after one month (Fig 2) and the patient is now 15 month old in normal growth and development

METHODS

Samples of pleural effusion were collected in sterile containers with addition of 300 ml water or by direct aspiration with a sterile syringe The pleural fluid was deep-

Table 1 *Amount of pleural effusion and contents of various lipids during the total period*

Day of treatment	Treatment	Pleural effusion (ml)	Triglyceride (mmol/l)	Total fatty acids (mmol/l)	Cholesterol (mmol/l)	Phospholipids (mmol/l)
Baseline	Human milk	ca 250	4 56	16 3	0 68	0 58
1–3	Parenteral	50				
4	-	10–15	1 14	6 5	1 73	1 13
5	-	40	0 45	5 3	1 80	1 11
6–7	-	230	0 31	4 5	1 59	0 93
8–9	Biosorbin®	200	1 90	10 9	1 53	1 05
10	-	25	3 80	18 2	1 35	1 35
11	-	45	5 2[b]	19 5[b]	0 25[b]	1 7[b]
12–17	-	300				
18–19	-	0				
20	-	70	3 3[b]	14 6[b]	1 0[b]	1 3[b]
21–24	-	0	-	-	-	-

Parenteral treatment consists of glucose 5% electrolytes aminoacids albumin were given four times during the first 27 days

[b] Calculated from dilutions of pleural fluid

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Submitted April 12 1976
Accepted July 9 1976

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Table 3 *Contents in serum of lipids during treatment*

Day of treatment	Treatment	Triglyceride (mmol/l)	Total fatty acids (mmol/l)	Cholesterol (mmol/l)	Phospholipids (mmol/l)
4	Parenteral	1 02	9 6	4 12	2 56
27	Biosorbin®	0 94		3 68	
50	-	0 95	8 6	3 24	2 20
90	-	0 49	7 8	3 40	2 07
180	Normal diet	0 84	11 6	3 38	2 28

* Parenteral treatment consists of glucose 5% electrolytes amino-acids albumin Bloodtransfusion were given 4 times during the first 27 days

can previously be found in litterature (3) In this report the effect of MCT on the pleural exudation was not recognized Lipid studies were not carried out

Two other studies of the effect of MCT in children with chylothorax have been reported (9 16) *In these patients the chylothorax* occured after thoracotomies, and the pleural effusion ceased after respectively 8 and 13 days MCT treatment

Lipid studies of the pleural fluid revealed *decrease in the concentration of triglyceride* and cholesterol concomitant with decrease in amount of pleural fluid In our case pleural effusion ceased after 14 days of MCT diet The pleural exudation tended to be smaller in the parenteral nutrition period than in the MCT period The cease of the pleural exudation might indicate a *spontaneous* recovery in accordance with natural cause of the disorder (18 19 26)

Therefore the lipid studies seems to be an important factor in evaluation of the MCT treatment The pronounced decrease in the triglyceride *and total fatty acids* concentrations in the pleural fluid during the *parenteral treatment period* suggests that intestinal lymph comprises a considerate part of the chyle in spontaneous neonatal chylothorax This theory is supported *by the fact that chylo*microns was not detected in this period Absence of changes in the cholesterol and phospholipid concentrations can be explained by the fact that cholesterol and phospholipid only exist in small amounts *in the membrane* of the chylomicrons (27)

During the *MCT treatment* the concentrations *of triglycerides and total fatty acids in*crease to levels seen on admission when the child *was feeded with human milk* These findings indicate that the administered Biosorbin in part is resorbed via the intestinal lymph *The small amount of long chained* essential fatty acids in Biosorbin could be one reason for this In the previous reports more than 95% of the fat content was comprised of MCT (9 16) We were prepared for a prolonged period of treatment and for this reason we used Biosorbin® formula containing essential fatty acid vitamins proteins etc

Previous reports describing favourable effects of MCT diet all deal with patients having posttraumatic chylothorax (9 11 16) while Brodman et al (3) did not observe any effect of MCT treatment in spontaneous neonatal *chylothorax The fact that the fat content in* the pleural fluid is significantly higher in posttraumatic chylothorax (18 26) may be due to a different pathogenesis in the cases and can possibly explain the lack of effect of MCT treatment in spontaneous neonatal chylothorax

We find like others (3 17) that rational treatment of patients with *spontaneous neonatal chylothorax* should consist of continuous *drainage of thorax—or repeated thoracocentesis*—combined with a parenteral nutrition with a sufficient supplement of calories proteins vitamins liquid and essential fatty acids, whereas MCT formula containing 87½% of the fat as MCT seems without effect

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Submitted April 12 1976
Accepted July 29 1976

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Table 3 *Contents in serum of lipids during treatment*

Day of treatment	Treatment	Triglyceride (mmol/l)	Total fatty acids (mmol/l)	Cholesterol (mmol/l)	Phospholipids (mmol/l)
4	Parenteral[a]	1 02	9 6	4 12	2 56
27	Biosorbin®	0 94		3 68	
50	-	0 95	8 6	3 24	2 20
90	-	0 49	7 8	3 40	2 07
180	Normal diet	0 84	11 6	3 38	2 28

[a] Parenteral treatment consists of glucose 5% electrolytes amino-acids albumin Bloodtransfusion were given 4 times during the first 27 days

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